PB84-138973

In vitro and In vivo Mutagenicity Studies of Environmental Chemicals

SRI International, Menlo Park, CA

Prepared for

Health Effects Research Lab.
Research Triangle Park, NC

Jan 84

U.S. Department of Commerce
National Technical Information Service
NTIS

PB84-138973

EPA-600/1-84-003
January 1984

IN VITRO AND IN VIVO MUTAGENICITY STUDIES OF ENVIRONMENTAL CHEMICALS

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NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

TECHNICAL REPORT DATA

(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/1-84-003	2.	3. RECIPIENT'S ACCESSION NO. PB84 138973
4. TITLE AND SUBTITLE IN VITRO AND IN VIVO MUTAGENICITY STUDIES OF ENVIRONMENTAL CHEMICALS		5. REPORT DATE January 1984 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) D.C.L. Jones, V.F. Simmon, K.E. Mortelmans, A.D. Mitchell, E.L. Evans, E.S. Riccio, D.E. Robinson, and B.A. Kirkhart		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS SRI International Menlo Park, CA		10. PROGRAM ELEMENT NO. CCAE1A 11. CONTRACT/GRANT NO. [illegible]
12. SPONSORING AGENCY NAME AND ADDRESS Office of Research and Development Health Effects Research Laboratory Environmental Protection Agency Research Triangle Park, NC 27711		13. TYPE OF REPORT AND PERIOD COVERED 14. SPONSORING AGENCY CODE EPA-600/11

15. SUPPLEMENTARY NOTES

Salmonella

16. ABSTRACT

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Seventy-nine compounds were evaluated for mutagenicity in one or more of 11 test systems: S. typhimurium plate incorporation assay; E. coli WP-2 reverse mutation assay; S. cerevisiae D3 mitotic recombination assay; S. cerevisiae D7 assays; E. Coli, B. subtilis, and S. typhimurium relative toxicity assays; siter-chromatid exchange in Chinese hamster ovary cells assay; L5178Y mouse lymphoma cell forward mutation assay; unscheduled DNA synthesis assay; mouse micronucleus assay; Drosophila sex-linked recessive lethal assay; and mouse dominant lethal assay.

The data from the evaluation of 41 pesticides and 10 industrial chemicals are presented in this report. Qualitative interpretations of these data and of data obtained under a previous contract, including those for an additional 28 pesticides, are summarized.

Saccharomyces

Bacillus

Escherichia

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT Release to Public	19. SECURITY CLASS (This Report) Unclassified	21. NO. OF PAGES 583
	20. SECURITY CLASS (This page) Unclassified	22. PRICE

DISCLAIMER

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new manmade environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, nonionizing radiation, environmental carcinogenesis, and the toxicology of pesticides as well as other chemical pollutants. The Laboratory participates in the development and revision of air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is primarily responsible for providing the health basis for nonionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

Many pesticide chemicals in commercial use today have not been adequately investigated for their potential mutagenic hazard. In view of the public's increasing concern about the possible pollution of the environment by chemicals, widely used pesticides must therefore be evaluated.

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Health Effects Research Laboratory

ABSTRACT

The objectives of this project were to evaluate the mutagenicity of various compounds, mostly pesticides, using microbial and mammalian cell *in vitro* techniques, as well as *in vivo* techniques in *Drosophila* and mice, and to further develop and refine these procedures for application as test batteries.

Seventy-nine compounds were evaluated for mutagenicity in one or more of 11 test systems: *S. typhimurium* plate incorporation assay; *E. coli* WP-2 reverse mutation assay; *S. cerevisiae* D3 mitotic recombination assay; *S. cerevisiae* D7 assays; *E. coli*, *B. subtilis*, and *S. typhimurium* relative toxicity assays; sister-chromatid exchange in Chinese hamster ovary cells assay; L5178Y mouse lymphoma cell forward mutation assay; unscheduled DNA synthesis assay; mouse micronucleus assay; *Drosophila* sex-linked recessive lethal assay; and mouse dominant lethal assay.

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CONTENTS

Foreword iii
Abstract iv
Acknowledgments ix

INTRODUCTION 1

TEST SYSTEMS--CHARACTERISTICS AND METHODS 4

Microbiological Assays 4

- General 4
- *Salmonella typhimurium* Plate Incorporation Assay (Ames Test) 4
  - Background 4
  - Method 5
  - Criteria for Interpretation 7
- *Escherichia coli* WP2 Assay 7
  - Background 7
  - Method 7
  - Criteria for Interpretation 8
- *Saccharomyces cerevisiae* D3 Mitotic Recombination Assay 9
  - Background 9
  - Method 9
  - Criteria for Interpretation 11
- *Saccharomyces cerevisiae* D7 Assay 11
  - Background 11
  - Method 12
  - Criteria for Interpretation 13
- *Escherichia coli* polA and *Bacillus subtilis* rec assays 14
  - Background 14
  - Method 15
  - Criteria for Interpretation 15

CONTENTS (cont'd)

Salmonella typhimurium Relative Toxicity Assay 16

- Background 16
- Method 16
- Criteria for Interpretation 17

Mammalian Cell Assays 18

Sister Chromatid Exchange in Cultured Chinese Hamster Ovary Cells 18

- Background 18
- Method 18
- Criteria for Interpretation 20

L5178Y $TK^{+/-}$ Mouse Lymphoma Forward Mutation Assay 20

- Background 20
- Method 21
- Criteria for Interpretation 22

Unscheduled DNA Synthesis Assay in WI-38 Cells 23

- Background 23
- Method 24
- Criteria for Interpretation 25

In Vivo Assays 26

Mouse Micronucleus Test 26

- Background 26
- Method 26
- Data Manipulation and Presentation 27
- Criteria for Interpretation 29

Drosophila Sex-Linked Recessive Lethal Assay 29

- Background 29
- Method 30
- Criteria for Interpretation 33

CONTENTS (cont'd)

RESULTS AND DISCUSSION 34

General 34

Pesticides 44

Acephate 44
Azinphos Methyl 62
Benomyl 87
Botran 102
Bromacil 111
sec-Butylamine (2-AB) 129
sec-Butylamine (2-AB)+H_3PO_4 137
Cacodylic Acid 145
Captan 165
Chlorpyrifos 178
Crotoxyphos 182
2,4D and 2,4DB Acids 199
Demeton 204
Diallate 222
Dicamba 239
m-Dichlorobenzene 242
o-Dichlorobenzene 248
p-Dichlorobenzene 255
Dinoseb 264
Disulfoton 268
DSMA 286
Folpet 289
Formetanate Hydrochloride 301
Malathion 309
Methomyl 312
Monocrotophos 314
Monuron 341
MSMA 359
Parathion 361
Parathion-Methyl 366
Pentachlorophenol 382
Phorate 389
Propanil 391
Rotenone 394
Simazine 398
2,4,5T 416
Triallate 424
Trichlorfon 440
Trifluralin 460
Vegadex 462

CONTENTS (cont'd)

Industrial Chemicals 468

- Acetonitrile 468
- Chlorobenzene 483
- Coal Tar (Flaked Pitch) 489
- Coal Tar (Bitumastic Component A) 498
- Coal Tar (Bitumastic Component B) 507
- Coal Tar (Bitumastic Component A + B) 516
- Ethanol 525
- Methanol 533
- Toluene 541
- Vinyl Fluoride..560

REFERENCES 568

ACKNOWLEDGMENTS

The diversity of disciplines and efforts required for this project is reflected in the personnel who contributed their talents and dedication. For the first year of this 34-month effort, Dr. Vincent F. Simmon, then Director, Microbial Genetics Department, was responsible for the overall project and for the microbial testing. During the remainder of the project, Dr. David C. L. Jones, Director, Toxicology Laboratory, was responsible for the overall project, and Dr. Kristien E. Mortelmans, Director, Microbial Genetics Department, supervised the microbial testing, which was performed by Edward S. Riccio, Gregory F. Shepherd, Mary V. Peirce, and Anne L. Pomeroy.

The mammalian cell mutagenesis testing was supervised by Dr. Ann D. Mitchell, Director, Biochemical Genetics Department. Mary M. Jotz conducted the mouse lymphoma testing, assisted by Douglas E. Rundle, Ronald L. Coleman, and Lynn S. Beckeart. Douglas E. Robinson performed the unscheduled DNA synthesis test, assisted by Martha L. Hay-Kaufman. Dr. Elizabeth L. Evans conducted the sister chromatid exchange assays, assisted by Marjorie L. Fong, Karen K. Yamamoto, Patricia A. McAfee, and Barbara L. Stewart, and supervised the *Drosophila* testing, assisted by Jennifer L. White and G. Ann Snyder. Barbara A. Kirkhart conducted the micronucleus tests. The extensive secretarial effort involved in preparing this report was provided by Birgit Westphal.

INTRODUCTION

The Federal Insecticide, Fungicide, and Rodenticide Act designates the Environmental Protection Agency as the governmental body responsible for the safety of all pesticides used in the United States. Subsequently, the Federal Environmental Pesticide Control Act (PL 92-516) strengthened EPA's regulatory responsibilities in the area of pesticides to include intra- as well as inter-state commerce.

To be federally registered, a pesticide must have been determined not to be hazardous to health or to the environment when used according to its labeling restrictions. Thus, in accordance with new law as well as with specific directives included in Public Law 93-135, 1973, EPA is now conducting a thorough review of the implications of using alternative chemicals, including older registered pesticides, for pest control.

In the pesticide review process, EPA emphasizes development of scientific criteria for evaluating the safety of compounds substituted for those pesticides found to be hazardous. In addition to review and evaluation of the literature on pesticides and maintenance of liaison with industry and academia, the strategy program includes laboratory studies to obtain additional data. One of these laboratory programs is directed toward gathering mutagenesis data on a selected number of compounds.

EPA's program is responsive to one of the recommendations included in the President's Scientific Advisory Committee Report of September 1973, *Chemicals and Health*. In that document, the Committee recommended that "Regulatory agencies should take steps to insure that new scientific data raising the possibility of new or extended hazards from chemicals in use are subject to careful process of scientific review for merit interpretation."

Development of methods for evaluating the mutagenic hazard of chemical compounds has advanced markedly in the last few years. In contrast to the undefined empirical tests used a short time ago, procedures now available can detect chromosome breaks, DNA damage, and mutational events caused by chemical stress. Mutant strains of microorganisms and mammalian cells are effective *in vitro* systems for reliable detection of genotoxic agents.

Many pesticide chemicals in commercial use today have not been adequately investigated for their potential mutagenic hazard. In view of the public's increasing concern about possible pollution of the environment by chemicals, the widely used pesticides must be evaluated.

Under contract to the EPA, SRI International evaluated the mutagenicity of a variety of compounds using microbial and mammalian cell *in vitro* techniques as well as *in vivo* techniques in *Drosophila* and in mice. Simultaneously, these test procedures were further developed and refined.

Efforts under this contract focused on two minor areas and on three major areas.

The first minor area was a series of experiments to obtain data on the size of mutant L5178Y mouse lymphoma cell colonies, using an Artek Model 880 colony counter system. The data were provided to the EPA in December 1980 for evaluation, and this area is not considered in this report.

The second minor area was the testing of nine compounds under subcontract from SRI to Microbiological Associates. The results of these tests have been reported by the subcontractor directly to the EPA, and are not considered in this report.

The first major area was SRI's participation in the International Program for the Evalution of Short-term Tests for Carcinogenicity. A number of laboratories evaluated 42 coded compounds, including known carcinogens, known noncarcinogens and unknowns, using a variety of test systems. After all test results had been reported, the compounds were decoded and the results were discussed by representatives of the participating laboratories and the sponsoring agencies in October 1979. The test systems used by SRI in this interlaboratory study were the *Salmonella* microsome assay (plate incorporation), sister chromatid exchange (SCE) in cultured Chinese hamster cells, forward mutation frequency in L5178Y mouse lymphoma cells, unscheduled DNA synthesis in human fibroblasts, and the mouse micronucleus test. The findings of the study have been presented elsewhere (1) and are not included in this report.

The second major area was the evaluation of 94 coded Diesel fractions as part of another interlaboratory comparison. The test systems employed were the *Salmonella* microsome assay, recombinogenesis in *Saccharomyces cerevisiae* D3, and the SCE and L5178Y mammalian cell assays. After all test results had been reported, the fractions were decoded and the results were discussed at a symposium by representatives of the participating laboratories and the EPA. The findings of the study have been published elsewhere (2) and/or submitted to the EPA in separate reports during the course of this contract, and are not included in this report.

The third major area was the evaluation of 41 pesticides and 10 industrial chemicals using 11 assay systems. Separate reports for each of the test systems for each of these compounds were submitted to the EPA as each test was completed.

In accordance with the terms of the contract, this final report includes a compilation of all quantitative data and a description of the test results for each of the 51 chemicals evaluated under the present contract, together with a summary table listing the qualitative interpretation of the results. In addition, the summary table includes the qualitative interpretations of the results of testing done under a previous contract, where 27 of the present 51 chemicals and 28 other chemicals were evaluated.

The qualitative interpretations for the 79 chemicals are based on a review of all of the data generated under both contracts, using the criteria described in the methods section of this report wherever possible. In some cases, this process has resulted in changes in the qualitative interpretations as compared with those in previous reports. For example, in previous reports, results for the relative toxicity studies in *E. coli* and *B. subtilis* were scored as positive when there was a clear compound effect, or as negative when there was not. In the present evaluation, positive or negative interpretation was limited to clear effect or no-effect results, with any other finding interpreted as inconclusive. Those cases where retesting and/or reevaluation was done are specified in the text and in the summary table.

TEST SYSTEMS--CHARACTERISTICS AND METHODS

This section describes each of the eleven test systems in terms of genetic background, methods used, and criteria for interpretation of the results. Although specific methods varied among the tests, certain principles were common to all of them: concurrent controls (positive, negative and/or solvent), preliminary range-finding experiments to establish doses and to select solvents, and inclusion of an external metabolic activation system when appropriate.

As noted above, many of the test procedures used were in a developing state during the course of this project. The following methods are those in use at the end of the project. The specific details of the test methods for each compound for each test system have been included in the individual compound reports submitted as each test was completed.

Microbiological Assays

General

Metabolic Activation System

Some carcinogenic chemicals (e.g., of the aromatic amine type or the polycyclic hydrocarbon type) are inactive unless they are metabolized to active forms. In animals and man, an enzyme system in the liver or other organs (e.g., lung or kidney) is capable of metabolizing a large number of these chemicals to carcinogens. Some of these intermediate metabolites are very potent mutagens in many *in vitro* short-term tests. The *S. typhimurium*, *E. coli* WP-2, and *S. cerevisiae* D3 and D7 tests on all compounds were carried out both in the absence and in the presence of a metabolic activation system. The metabolic activation mixture for each experiment consisted of, for 10 ml: 1.00 ml of S-9 fraction, 0.20 ml of $MgCl_2$ (0.4 M) and KCl (1.65 M), 0.05 ml of glucose-6-phosphate (1 M), 0.40 ml of NADP (0.1 M), 5.00 ml of sodium phosphate buffer (0.2 M, pH 7.4) and 3.35 ml of H_2O.

Salmonella typhimurium Plate Incorporation Assay (Ames Test)

Background

The *Salmonella typhimurium* strains used at SRI are all histidine auxotrophs by virtue of mutations in the histidine operon. When these histidine-dependent cells are grown on minimal medium agar plates containing a trace of histidine, only those cells that revert to histidine independence (*his^+*) are able to form colonies. The small amount of histidine allows all the plated bacteria to undergo a few divisions; in many cases, this growth is essential for mutagenesis to occur. The *his^+* revertants are easily visible as colonies against the slight background growth. The spontaneous mutation frequency of each strain is relatively constant (3,4), but when a mutagen is added to the agar, the mutation frequency is increased, usually in a dose-related manner.

The tester strains used at SRI are TA1535, TA1537, TA1538, TA98, and TA100. They were obtained from Dr. Bruce Ames of the University of California at Berkeley (5). In addition to having mutations in the histidine operon, all the indicator strains have a mutation (rfa) that leads to a defective lipopolysaccharide coat; they also have a deletion that covers genes involved in the synthesis of the vitamin biotin (bio) and in the repair of ultraviolet (uv)-induced DNA damage (uvrB). The uvrB mutation causes decreased repair of some types of chemically or physically damaged DNA and thereby enhances the strains' sensitivity to some mutagenic agents. Strain TA1535 is reverted to his^+ by many mutagens that cause base-pair substitutions. Strain TA100 is derived from TA1535 by the introduction of the resistance transfer factor, plasmid pKM101. This plasmid is believed to cause an increase in error-prone DNA repair that leads to many more mutations for a given dose of most mutagens (6). In addition, plasmid pKM101 confers resistance to the antibiotic ampicillin, which is a convenient marker to detect the presence of the plasmid in the cell (7). The presence of this plasmid also makes strain TA100 sensitive to some frameshift mutagens [e.g., ICR-191, benzo(a)pyrene, aflatoxin B_1, and 7,12-dimethylbenz(a)anthracene]. Strains TA1537 and TA1538 are reverted by many frameshift mutagens. Strain TA98 is derived from TA1538 by the addition of the plasmid pKM101, which makes it more sensitive to some mutagenic agents (5).

All indicator strains are kept at 4°C on minimal agar plates supplemented with an excess of biotin and histidine. The plates with the plasmid-carrying strains also contain ampicillin (25 µg/ml) to ensure stable maintenance of the plasmid pKM101. New stock culture plates are made every four to six weeks from single colony isolates that have been checked for their genotypic characteristics (his, rfa, uvrB, bio) and for the presence of the plasmid. For each experiment, an inoculum from the stock culture plates is grown overnight at 37°C in nutrient broth (Oxoid, CM67).

Method

To a sterile 13 x 100 mm test tube placed in a 43°C heating block, we add in the following order:

(1) 2.00 ml of 0.6% agar*
(2) 0.05 ml of indicator organisms
(3) 0.50 ml of metabolic activation mixture (if appropriate)
(4) 0.05 ml of a solution of the test chemical.

---

*The 0.6% agar contains 0.05 mM histidine, 0.05 mM biotin, and 0.6% NaCl.

This mixture is stirred gently and then poured onto minimal agar plates.* After the top agar has set, the plates are incubated at 37°C for 2 days. The numbers of his$^+$ revertant colonies are recorded.

The solvent of choice is distilled water or 0.067 M phosphate buffer (pH 7.4). Chemicals that do not dissolve in an aqueous solvent are dissolved in dimethylsulfoxide (DMSO) or other suitable solvent. Test chemicals are prepared on the day they are tested. Serial dilutions are made from initial stock. Concentrations are expressed as μg/plate.

Concurrent negative, solvent, and positive controls (known mutagens) controls are also run. The following positive control chemicals are used:

- Sodium azide for the base-pair substitution mutants TA1535 for TA100.
- 9-Aminoacridine for the frameshift mutant TA1537.
- 2-Nitrofluorene for the frameshift mutants TA1538 and TA98.
- 2-Aminoanthracene for all tester strains, in the presence of metabolic activation.

A preliminary range-finding test is run with strain TA100 on all chemicals to determine a suitable nontoxic range of concentrations. Each chemical is tested at a minimum of six concentrations, with the highest nontoxic concentration tested being 10 mg/plate unless specific solubility dictates a lower upper limit. The concentrations tested are reduced appropriately for toxic chemicals. Ideally, at least four nontoxic concentrations are tested.

Compounds are tested at six levels, covering a wide range of concentrations, in the presence and in the absence of mammalian liver metabolic activation system. The highest concentration used depends on the solubility and toxicity of the test compound. All assays are repeated at least once on a separate day.

Highly volatile chemicals (e.g., vinyl fluoride) are difficult to test in the normal assay procedures because of loss of material during the incubation step. We have developed a special procedure for testing this type of compound. The Salmonella plates are prepared as described for the assays in agar, but no test chemical is added. The plates, the lids having been removed, are placed side by side on a perforated shelf in a 9-liter desiccator. After evacuation of the air, a known volume of the liquid test chemical is added from a syringe to a glass petri plate placed in the center of and attached to the bottom of the shelf. The pressure inside is then equilibrated with room air via a sterile cotton plug, the desiccator is sealed and placed on a magnetic stir-plate in a room maintained at 37°C. A magnetic stirrer with vanes, placed in the base of each desiccator, ensures adequate dispersion of the chemical. After incubation for 8 hours, the plates are removed from the desiccators, their lids are replaced, and they are incubated at 37°C for an additional 40 hours. The number of *his*$^+$ revertants is counted and recorded.

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*Minimal agar plates consist of, per liter: 15 g of agar, 10 g of glucose, 0.2 g of $MgSO_4 \cdot 7H_2O$, 2 g of citric acid monohydrate, 10 g of K_2HPO_4, and 3.5 g of $NaNH_4HPO_4 \cdot 4H_2O$.

Criteria for Interpretation

The actual numbers of colonies observed on the plates are reported; no statistical analysis is performed.

Positive. A compound is considered a mutagen when it produces a reproducible dose-related increase in the number of revertants in one or more strains. This increase should occur on at least three concentration levels.

Negative. A compound is considered a nonmutagen when no dose-related increase in the number of revertants is observed in at least two independent experiments. For nontoxic compounds, the maximum concentration tested is 10 mg/plate (unless dictated otherwise by solubility problems). For toxic compounds, only the highest concentration tested should show evidence of toxicity.

Inconclusive. When a compound cannot be identified clearly as a mutagen or nonmutagen, the results are classified as inconclusive.

Escherichia Coli WP2 Assay

Background

E. coli strain WP2 is a tryptophan auxotroph (*trp*) by virtue of a base-pair substitution mutation in the tryptophan operon. In addition, it is deficient in the repair of some physically or chemically induced DNA damage (*uvrA*) (8). This *uvrA* mutation makes the strain more sensitive to certain mutagens.

The *E. coli* WP2 (9) strains used at SRI were obtained originally from Dr. D. McCalla. The strains are kept at 4°C on yeast-complete agar plates. New stock cultures are made every four weeks from single-colony isolates. For each experiment, an inoculum from the stock culture plates is grown overnight at 37°C in nutrient broth (Oxoid CM67).

Method

To a sterile 13 x 100-mm test tube placed in a 43°C heating block, we add in the following order:

(1) 2.00 ml of 0.6% agar*
(2) 0.05 ml of indicator organisms
(3) 0.50 ml of a metabolic activation mixture (optional)
(4) 0.05 ml of a solution of the test chemical.

This mixture is stirred gently and then poured onto minimal agar plates† supplemented with a 1% (v/v) oxoid nutrient broth. After the top agar has set, the plates are incubated at 37°C for two days. The number of trp^+ revertants is counted and recorded.

The solvent of choice is distilled water or 0.067 M phosphate buffer (pH 7.4). Chemicals that do not dissolve in an aqueous solvent are dissolved in DMSO or other suitable solvent. Test chemicals are always prepared on the day they are tested. Serial dilutions are made from initial stock. Concentrations are expressed as µg/plate.

Concurrent negative (solvent) and positive (known mutagen) controls are run with each experiment. The positive control chemicals are [2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide] (AF-2) (0.1 µg/plate) without metabolic activation and 2-aminoanthracene (10.0 µg/plate) with metabolic activation.

A preliminary range-finding test is run on all chemicals to determine a suitable nontoxic range of concentrations. Each chemical is tested at a minimum of six concentrations, with the highest nontoxic concentration tested being 10 mg/plate unless specific solubility dictates a lower upper limit. The concentrations tested are reduced appropriately for toxic chemicals. Ideally, at least four nontoxic concentrations are tested.

Compounds are tested at six levels, covering a wide range of concentrations, in the presence and in the absence of a mammalian liver metabolic activation system. The highest concentration used depends on the solubility, toxicity, and availability of the test compound. All assays are repeated at least once on a separate day.

Volatile chemicals are tested in a dessicator, using a modified exposure technique essentially as described above for the *S. typhimurium* Plate Incorporation Assay.

Criteria for Interpretation

A mutagenic response is defined as a dose-related increase in the number of trp^+ revertant colonies per plate. The actual numbers of colonies observed on the plates are reported. An experiment is considered invalid when control values are not within acceptable limits or when three or more of the six concentrations selected exhibit toxicity. In these cases, the experiment is repeated, with appropriately adjusted concentration levels.

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*The 0.6% agar contains 0.05 mM histidine, 0.05 mM biotin, and 0.6% NaCl.

†Minimal agar plates consist of, per liter: 15 g of agar, 10 g of glucose, 0.2 G of $MgSO_4 \cdot 7H_2O$, 2 g of citric acid monohydrate, 10 g of K_2HPO_4, and 3.5 g of $NaNH_4HPO_4 \cdot 4H_2O$.

Positive. A compound is considered a mutagen when it produces a dose-related increase in the number of revertants. This increase should occur over at least three concentration levels.

Negative. A compound is considered a nonmutagen when no dose-related increase in the number of revertants is observed in at least two independent experiments. For nontoxic compounds, the maximum concentration tested should be 10 mg/plate (unless dictated otherwise by solubility problems). For toxic compounds, only the highest concentration tested should show evidence of toxicity.

Inconclusive. When a compound cannot be identified clearly as a mutagen or nonmutagen, the results are classified as inconclusive.

Saccharomyces Cerevisiae D3 Mitotic Recombination Assay

Background

The yeast *S. cerevisiae* D3 is a diploid microorganism heterozygous for a mutation leding to a defective enzymes in the adenine-metabolizing pathway (10). When grown on medium containing adenine, cells homozygous for this mutation produce a red pigment. These homozygous mutants can be generated from the heterozygotes by mitotic recombination. The frequency of this recombinational event may be increased by incubating the organisms with various recombinogenic agents. The recombinogenic activity of a compound or its metabolite is determined from the number of red-pigmented colonies appearing on test plates (11).

The *S. cerevisae* D3 strain was obtained from F. K. Zimmerman. The stock culture is stored at 4°C. For each experiment, broth containing 0.05% $MgSO_4$, 0.15% KH_2PO_4, 0.45% $(NH_4)_2SO_4$, 0.35% peptone, 0.5% yeast extract, and 2% dextrose is inoculated with a loopful of the stock culture and incubated overnight at 30°C with shaking.

Method

An overnight culture is centrifuged and the cells are resuspended at a concentration of 10^8 cells/ml in 0.067 mM phosphate buffer (pH 7.4). To a sterile test tube is added:

- 1.00 ml of the suspended culture
- 0.50 ml of either the metabolic activation mixture or buffer
- 0.20 ml of the test chemical
- 0.30 ml of buffer.

Several concentration levels of the test chemical are used in each experiment, and appropriate controls are included. In this report, concentrations are expressed in percent. Conversion to µg/ml can be done by multiplying the percent value by 10^4.

The suspension mixture is incubated at 30°C for 4 hours on a roller drum. The sample is then diluted serially in sterile physiologic saline, and 0.2 ml of the 10^{-5} and 10^{-3} dilutions is spread on plates containing the same ingredients as the broth plus 2.0% agar; five plates receive the 10^{-3} dilution and three plates receive the 10^{-5} dilution. The plates are incubated for 2 days at 30°C, followed by 2 days at 40°C to enhance the development of the red pigment indicative of adenine-deficient homozygosity. Plates containing the 10^{-3} dilution are scanned with a dissecting microscope at 10X magnification, and the number of mitotic recombinants (red colonies or red sectors) are recorded.

Depending on the test material's solubility characteristics, the solvent of choice is water, DMSO, ethanol, or acetone. If more than one solvent can be used, preference will be given in the order listed here. If information on the test chemical's solubility is not available, a preliminary solubility test is performed.

Once the solvent is selected and its solubility is verified, a series of dilutions of the test sample is made. The same volume of sample/solvent is added to each test tube (usually 0.2 ml), and the concentration of each dilution is adjusted appropriately. The test concentrations selected depend on the solubility and toxicity of the sample.

For the solvent control preparations we add to a sterile test tube:

- 1.00 ml of the overnight broth culture
- 0.50 ml of either metabolic activation mixture or buffer
- 0.20 ml of the solvent used
- 0.30 ml of buffer.

For the positive control preparations, 1,2,3,4-diepoxybutane is substituted for the test chemical at a concentration of 0.025%.

A preliminary experiment is performed to determine a suitable range of concentration levels for final testing. Several levels are chosen (at least four), with the concentration of compound in the test tube ranging from 1 to 5%.

Final testing consists of two experiments performed on separate days. Additional testing is done if the results are not reproducible or if a chemical is toxic or marginally recombinogenic.

Five concentrations of the chemical are usually tested with and without metabolic activation. If no toxicity or no recombinant activity is obtained in the preliminary experiment, the same concentration range is used for final testing. When a compound is toxic, the range is shifted down until about 50% final toxicity is reached. The concentrations used cover a narrow range expected to give a recombinogenic dose response. Nontoxic recombinogenic substances are retested using a concentration range that gives a recombinogenic dose response, preferably in the linear portion of the curve. When the positive control compound, 1,2,3,4-diepoxybutane, does not elicit recombinogenic activity, the experiment is considered invalid.

Volatile chemicals are tested in a desiccator, using a modified exposure technique, essentially as described above for the S. typhimurium Plate Incorporation Assay.

Criteria for Interpretation

The surviving fraction of organisms is determined from the total number of colonies appearing on the plates of the 10^{-5} dilution. The number of mitotic recombinants per 10^5 survivors is calculated.

Positive. A positive response in this assay is indicated by a dose-related increase of more than threefold in the absolute number of mitotic recombinants per milliliter and in the relative number of mitotic recombinants per 10^5 survivors.

Negative. If no recombinogenic activity is obtained in any of the assays performed, the test results are considered negative.

Inconclusive. When positive results are not dose-related, they are considered inconclusive.

Saccharomyces Cerevisiae D7 Assay

Background

S. cerevisiae D7 is a diploid yeast strain that can be used to study induction of mitotic crossing-over, mitotic gene conversion, and reverse mutation (12, 13). The frequency of these events may be increased by incubating the organisms with various mutagenic or recombinogenic agents.

Mitotic crossing-over. S. cerevisiae D7 is heterozygous for a mutation leading to a defective enzyme in the adenine-metabolizing pathway. When grown on medium containing adenine, cells homozygous for this mutation produce pink and red twin-sectored colonies of the genotype ade2-40/ade2-40 (deep red) and ade2-119/ade2-119 (pink) from the original heteroallelic condition, ade2-40/ade2-119, which forms white colonies. These homozygous mutants can be generated from heterozygotes by mitotic crossing-over, and the frequency of this event is determined by the numbers of twin-sectored colonies appearing on the plates.

Also detected by this strain that is heteroallelic for ade2-40/ade2-119 are colonies that are pink, red, white and pink, and red and white. These additional colonies appear more frequently than the twin-sectored colonies and may result from a variety of events--such as point mutation, gene conversion, chromosomal deletions, and aneuploidy. No effort is made to distinguish the different phenotypic aberrants or their causes--they are merely grouped as total aberrants.

Gene conversion. S. cerevisiae D7 is heterozygous for a mutation leading to a specific growth requirement. The heterozygous condition, trp5-12/trp5-27, leads to the requirement of exogenous tryptophan for growth. Conversional transfer of the intact region of one mutant allele to replace the defective nucleotide sequence in the other mutant allele will restore a true wild-type genotype, which allows growth in the absence of tryptophan. Thus, by plating the test substance on defined medium that lacks tryptophan, the frequency of mitotic gene conversion can be measured by the numbers of colonies not requiring tryptophan that appear on the selective media plates.

Reverse mutation. S. cerevisiae D7 is homozygous at the ilvi92/ilvi92 locus and requires isoleucine for growth. The isoleucine requirement caused by the homozygous condition can be alleviated by true reverse mutation and by allele-nonspecific suppressor mutation. Mutation induction can be followed by the appearance of colonies not requiring isoleucine on selective media. When the test material is plated on medium that lacks isoleucine, only the mutants are capable of growing. The frequency of the reverse mutation can therefore be measured by the numbers of revertant colonies that appear on plates lacking isoleucine.

The S. cerevisiae D7 strain used at SRI was obtained from Dr. F. K. Zimmerman. It is stored at 4°C on yeast-complete media. For each experiment, broth containing 0.05% $MgSO_4$, 0.15% KH_2PO_4, 0.45% $(NH_4)_2SO_4$, 0.35% peptone, 0.5% yeast extract, and 2% dextrose is inoculated with a loopful of the stock culture and incubated overnight at 30°C with shaking.

Method

The overnight culture is centrifuged, and the cells are resuspended at a concentration of 10^8 cells/ml in 0.067 mM phosphate buffer (pH 7.4). To a sterile test tube is added:

- 1.00 ml of the resuspended culture
- 0.50 ml of either the metabolic activation mixture or buffer
- 0.20 ml of the test chemical
- 0.3 ml of buffer.

Several concentrations of the test chemical (up to 5%, w/v or v/v) are tested in each experiment, and the appropriate controls are included. The suspension mixture is incubated at 30°C for 4 hours on a roller drum. The sample is then diluted serially in sterile physiologic saline. In this report, concentrations are expressed in percent. Conversion to µg/ml can be done by multiplying the percent value by 10^4.

For detection of mitotic crossing-over, 0.2 ml of the 10^{-5} and 10^{-3} dilutions are added at 2.0 ml of top agar and poured onto plates containing the same ingredients as the broth plus 1.5% agar; five plates receive the 10^{-3} dilution and three plates receive the 10^{-5} dilution. The plates are then incubated for 2 days at 30°C followed by 2 days at 4°C to enhance the development of the red pigment indicative of adenine-deficient homozygosity. Plates containing the 10^{-3} dilution are scanned with a dissecting microscope at 10X magnification, and the numbers of mitotic recombinants (pink/red twin-sectored colonies) and aberrants (pink, red, pink and white, red and white) are recorded.

For detection of mitotic gene conversion, 0.2 ml of the 10^{-1} dilution is added to 2.0 ml of top agar and plated on synthetic minimal medium plates that lack tryptophan. These plates are incubated for 5 days at 30°C, and the revertant colonies are then counted.

For detection of reverse mutation, 0.2 ml of the test suspension (10^0 tubes) is added to 2.0 ml of top agar and plated on synthetic medium plates that lack isoleucine. These plates are incubated for 5 days at 30°C, and the revertant colonies are then counted.

The solvent of choice is sterile water. However, because the majority of organic compounds are not sufficiently water-soluble, particularly at higher concentrations, DMSO is also used routinely. Other suitable organic solvents are sometimes used.

Once the solvent is selected and its solubility is verified, a series of dilutions of the test sample is made. It is preferable that the same volume of sample/solvent be added to each test tube (usually 0.2 ml) and that the concentration of each dilution be adjusted appropriately. The test concentrations selected depend on the amount of sample available and its solubility and toxicity (if known).

For the negative controls, we add to a sterile test tube 1.00 ml of the overnight broth culture, 0.50 ml of either metabolic activation mixture or buffer, 0.20 ml of the solvent used, and 0.30 ml of buffer.

For the positive control, 1,2,3,4-diepoxybutane is substituted for the test chemical at a concentration of 0.025%.

A preliminary experiment is performed on most test samples to determine a suitable range of concentration levels for final testing. Several levels are chosen (at least four), with the concentration of compound in the test tube ranging from 1% to 5%.

Final testing consists of two experiments performed on separate days. Additional testing is done if the results are not reproducible or if a chemical is toxic or marginally positive.

Five concentrations of the chemical are usually tested with and without metabolic activation. If no toxicity or no genetic activity is obtained in the preliminary experiment, the same concentration range is used for final testing. When a compound is toxic, the concentration range is shifted downward until about 50% final toxicity is reached. Nontoxic recombinogenic substances are retested using a concentration range that gives a dose-response relationship, preferably in the linear portion of the curve.

When the positive control, 1,2,3,4-diepoxybutane, does not elicit the expected genetic activity, the experiment is considered invalid and is repeated.

Criteria for Interpretation

The surviving fraction of organisms is determined from the total number of colonies appearing on the plates of the the 10^{-5} dilution. The number of mitotic recombinants and the number of total aberrants per 10^5 survivors are calculated. The number of gene convertants is calculated per 10^6 survivors and the number of revertants is calculated per 10^7 survivors.

Positive. A positive response for mitotic crossing-over is indicated by a dose-related increase in the absolute number of mitotic recombinants and aberrants per milliliter as well as in the relative number of mitotic recombinant and aberrants per 10^5 survivors.

A positive response for mitotic gene conversion is indicated by a dose-related increase in the number of convertant colonies that do not require tryptophan, as well as the number of convertant colonies of 10^6 survivors.

A positive response for reverse mutation is indicated by a dose-related increase in the number of true revertant colonies that do not require isoleucine as well as by the number of revertant colonies per 10^7 survivors.

Negative. A compound is considered to give a negative result when no dose-related increase is observed in the number of mitotic recombinants, in the number of convertant colonies, and in the number of true revertant isoleucine-independent colonies.

Inconclusive. When a compound cannot be identified clearly as causing a positive or a negative response, the results are classified as inconclusive.

Escherichia coli polA and Bacillus subtilis rec Relative Toxicity Assays

Background

E. coli strain p3478 is a DNA polymerase-deficient (*polA*$^-$) derivative of strain W3110 (14). The enzyme DNA polymerase I is involved in resynthesizing DNA segments that have been damaged and excised (15). Because strain p3478 lacks DNA polymerase I, DNA damage (caused by physical or chemical agents that react with DNA) is not repaired as efficiently as it is in the parent strain. Exposure to DNA-damaging agents will usually inhibit the growth of p3478 more than that of W3110. Therefore, test compounds that are more toxic to p3478 than to W3100 are probably reacting with the DNA. Compounds that do not react with DNA are generally equally toxic (or nontoxic) to both strains. The ability of a test compound to induce DNA damage can thus be evaluated by comparing its relative toxicity (zone of inhibition of growth) in these two strains. We obtained *E. coli* strains W3110 and p3478 from Dr. H. Rosenkranz (16).

B. subtilis strain M45 is a recombination-deficient (*recA*$^-$) derivative of strain H17. Recombination is required for repair of damaged DNA. Strain M45 lacks the *recA* function, so damage caused by agents that react with DNA is not as efficiently repaired in M45 as it is in the parent strain H17. The ability of a test compound to induce DNA damage can be evaluated by comparing its relative (differential) toxicity to the *recA* (H17) and *recA*$^-$ (M45) strains. Compounds that do not react with DNA are generally equally toxic (or nontoxic) to both strains (17). *B. subtilis* strains H17 and M45 were obtained from Dr. T. Kada.

Method

For each experiment with each species, a 1-ml inoculum of a frozen (-80°C) stock culture is grown overnight at 37°C, with shaking, in broth consisting of 0.5% yeast extract, 0.35% peptone, 2% glucose, 0.05% $MgSO_4$, 0.15% KH_2PO_4, and 0.45% NH_4SO_4. To 2 ml of top agar (0.6% NaCl and 0.6% agar) is added 0.1 ml of the overnight bacterial culture (approximately 3×10^8 cells). The suspension is mixed and poured onto the surface of a plate containing the same ingredients as the broth plus 2% agar (25 ml). When the top agar has solidified, a sterile filter disc (6 mm in diameter) is placed in the center of the plate and inoculated with the test substance. Concentrations are expressed in mg/disc or plate. Plates are incubated at 37°C for 16 to 17 hours, and then the diameter of the zone of inhibition of growth is measured.

Depending on the test material's solubility characteristics, the solvent of choice is water, DMSO, ethanol, or acetone. If more than one solvent can be used, preference is given in the order listed here. If information on the test chemicals' solubility is not available, a preliminary solubility test is performed. Once the solvent is selected and its solubility is verified, a series of dilutions of the test sample is made. The test concentrations selected depend on the sample's solubility.

Methyl methanesulfonate is used as the positive control compound in this assay. The negative control compound is ampicillin, kanamycin, or chloramphenicol. They each induce equal zones of growth inhibition to repair-proficient and repair-deficient strains because their toxic effects are not due to DNA damage. A solvent control--without any chemical--is also used.

A preliminary experiment is performed on test samples to determine a suitable concentration range for final testing. Several concentration levels are chosen (at least three), covering a range of 5 to 30 microliters of the solution on the filter disc. Final testing consists of two experiments performed on separate days. Additional testing is done if the results of these experiments are not reproducible.

Criteria for Interpretation

The zones of growth inhibition, expressed in millimeters, constitute the data.

Positive. A chemical that reacts with and damages DNA gives rise to a differential toxicity response. The zone of growth inhibition is greater on the plates with the repair-deficient strain than on the plates with the repair-proficient strain.

Negative. A negative response is indicated by equal zones of growth inhibition on the plates with the repair-deficient and repair-proficient strains.

Inconclusive. A result is considered inconclusive when no toxicity (no growth inhibition) is observed on either the test plates with the repair-proficient strain. on the test plates with the repair-proficient strain.

Salmonella typhimurium Relative Toxicity Assay

Background

We obtained S. typhimurium strains SL4525 (rec^+) and SL4700 (rec^-) from Dr. Bruce Stocker of Stanford University; strains TA1978 ($uvrB^+$) and TA1538 ($uvrB^-$) were obtained from Dr. Bruce Ames of the University of California at Berkeley. These four strains have an rfa mutation that leads to a defective lipopolysaccharide coat, making them more permeable to many large molecules, thus enhancing the potential for genetic damage by some mutagenic agents.

Strain SL4700 is a recombination-deficient (rec^-) derivative of strain SL4525. Recombination is required for repair of some types of damaged DNA. Strain SL4700 lacks the rec function; thus, damage caused by agents that react with DNA is not as efficiently repaired in strain SL4700 as it is in strain SL4525 (18).

Strain TA1538, which lacks the uvrB gene, is derived from strain TA1978. The uvrB mutation causes decreased repair of some types of chemically or physically damaged DNA and thereby increases the strain's sensitivity to some mutagenic agents. Because strain TA1538 lacks the uvrB gene, genetic damage is not as efficiently repaired as it is in the parent strain TA1978 (5).

The ability of a test compound to induce DNA damage can be evaluated by comparing its differential toxicity to the repair-proficient and repair-deficient strains upon exposure to the test chemical. Compounds that cause a reproducibly larger zone of inhibition of growth to the repair-deficient strain than to the repair-proficient strain probably are reacting with the cell's DNA and are reported as giving positive results. Test compounds that do not react with the cell's DNA generally cause equal zones of inhibition of growth to both strains and are reported as giving negative results. When the test compound fails to exhibit any sign of toxicity (inhibition of growth), the experiment is declared a no-test because the results are inconclusive.

Method

For each experiment, a 1-ml inoculum of a frozen (-80°C) stock culture is grown overnight at 37°C, with shaking, in broth consisting of 0.5% yeast extract, 0.35% peptone, 2% glucose, 0.05% $MgSO_4$, 0.15% KH_2PO_4, and 0.45% NH_4SO_4. To 2 ml of top agar (0.6% NaCl and 0.6% agar) is added 0.05 ml of the overnight bacterial culture (approximately 3×10^8 cells). The suspension is mixed and poured onto the surface of a plate containing the same ingredients as the broth plus 2% agar (25 ml). When the top agar has solidified, a sterile filter disc (6 mm in diameter) is placed in the center of the plate and inoculated with the test substances. Concentrations are expressed as mg/disc or plate. Plates are incubated at 37°C for 16 to 17 hours; then the diameter of the zone of inhibition of growth is measured.

Several concentrations of the test substances are tested in duplicate. Methyl methanesulfonate is used as the positive control for the SL4525/SL4700 assay and 4-nitroquinoline-1-oxide is the positive control for the TA1978/TA1538 assay. The negative control is kanamycin, an antibiotic that exerts its toxic effect by means other than DNA damage. A solvent control (no chemical) is included in each experiment.

A preliminary experiment is performed on test samples to determine a suitable concentration range for final testing. Several concentration levels are chosen (at least three), covering a range of 5 to 30 microliters of the solution on the filter disc. Final testing consists of two experiments performed on separate days. Additional testing is done if the results of these experiments are not reproducible.

Criteria for Interpretation

The zones of growth inhibition, expressed in millimeters, constitute the data.

Positive. A chemical that reacts with and damages DNA gives rise to a differential toxicity response. The zone of growth inhibition is greater on the plates with the repair-deficient strain than on the plates with the repair-proficient strain.

Negative. A negative response is indicated by equal zones of growth inhibition on the plates with the repair-deficient and repair-proficient strains.

Inconclusive. A result is considered inconclusive when no toxicity (no growth inhibition) is observed on either the test plates with the repair-deficient strain or the test plates with the repair-proficient strain.

Mammalian Cell Assays

Sister Chromatid Exchange in Cultured Chinese Hamster Ovary Cells

Background

The induction of DNA lesions by chemical mutagens leads to the formation of sister chromatid exchanges (SCEs), which may be related to a recombinational or postreplicative repair of DNA damage (19-21). Exchanges between the two chromatids of each chromosome are observed in cells that have been grown in the presence of bromodeoxyuridine (BrdU) for two rounds of replication. Because of semiconservative DNA replication, such chromosomes possess one chromatid that is half BrdU-substituted and one that is fully substituted. These chromatids are differentially stained by the fluorescence-plus-Giemsa (FPG) techniques (22); hence, exposure to a chromosome-damaging agent results in an increased frequency of SCEs, revealed by the "harlequin" pattern of darkly- and lightly-staining chromatid segments.

The SCE technique is highly sensitive [illegible] mammalian cells, it can detect effects on chromosomes by direct-acting chemical mutagens (20-21), as well as by those that require metabolic activation (23, 24) at doses much lower than those required in other *in vitro* cytogenetic assays. Wolff (25) has reviewed the chemicals that have been shown to cause increases in SCE frequency. We have conducted studies (26, 27) to correlate the effects of mutagenic agents measured by the SCE technique, with the induction of unscheduled DNA synthesis and forward mutation observed in mammalian cells. Because of the sensitivity of the SCE technique, a doubling of the background SCE frequency at the 5% level of significance can be detected by analysis of only 20 cells per sample. Therefore, scoring of SCEs is more rapid than the enumeration of aberrations in other *in vitro* cytogenetic tests.

Method

SRI uses cultured Chinese hamster ovary (CHO) cells from the American Type Culture Collection (ATCC-CCL-61), Rockville, MD. Additional stocks of CHO cells are stored in a liquid-nitrogen freezer. The cells are maintained at an exponential growth rate in an atmosphere of 5% CO_2 at 37°C in McCoy's 5a medium with 15% fetal calf serum (FCS), 2 mM ℓ-glutamine, and 1% penicillin-streptomycin solution. This medium is also used during exposure of the cells to the compound and to BrdU. The cell cycle time is approximately 11 hours.

The positive control compounds are ethyl methanesulfonate, a compound that induces SCEs in the absence of a metabolic activation system, and dimethylnitrosamine, a compound that induces SCEs only with metabolic activation. The vehicle control preparation contains solvent diluted in culture medium. For assays using metabolic activation, the activation mixture is also added to the vehicle control preparation. The negative control preparations contain untreated culture medium for assays without metabolic activation and metabolic activation mixture for assays using metabolic activation. When the solvent is water, the negative and vehicle controls are equivalent.

For testing with metabolic activation, we use a preparation consisting of the 9000 x *g* supernatant (S-9) of a liver homogenate from adult male Fischer 344 rats weighing 150-180 g and given a single 500-mg/kg intraperitoneal injection of Aroclor 1254 five days before sacrifice. Determinations of Lowry protein (28), aryl hydrocarbon hydroxylase (29), and sterility are made on each new batch of S-9. The metabolic activation mixture conists of one part S-9 to nine parts of McCoy's 5a medium containing 2% fetal calf serum (FCS), 2 mM ℓ-glutamine, and 1% penicillin-streptomycin solution. Cofactors are added: 24 mg of NADP and 45 mg of sodium isocitrate per milliliter of S-9. This mixture is prepared fresh before use.

Preliminary cytotoxicity measurements are necessary to determine the most effective concentrations of each test compound. Since chemicals may affect the duration of the cell cycle and since treated cells must divide twice during exposure to BrdU to be evaluated for SCE induction, a series of five dilutions of the compound and positive, vehicle, and negative controls are used to duplicate the assay conditions with and without metabolic activation. Concentrations are expressed in µg/ml. After 2.5 hours in colchicine, cells are harvested at 24 hours. Chromosome preparations from these cultures are stained by the modified FPG technique (22), and the slides are examined by fluorescence microscopy to determine whether the metaphases have divided one, two, or three times during exposure to BrdU. The highest concentration of the compound selected for further testing will be one that is expected to permit enough cells to divide twice so that SCEs can be scored.

For the test itself, a modification of the procedures developed by Perry and Evans (20) and Stetka and Wolff (24) is used. Each compound is tested with and without metabolic activation, and duplicate samples of each compound concentration and of the positive, negative, and vehicle controls are tested. In tests performed without metabolic activation, the test compound is added simultaneously with 10^{-5} M BrdU in fresh complete McCoy's 5a medium, and the cells are grown in the dark at 37°C for 21.5 hours. Then the medium is replaced with fresh complete McCoy's 5a medium containing colchicine (0.4 µg/ml), and the cultures are incubated for 2.5 hours before they are harvested.

In tests performed with metabolic activation, the cell cultures are exposed to the test compound in the metabolic activation mixture for two hours (this shorter exposure time is used to preclude cytotoxic effects from the metabolic activation preparation). After exposure, the medium is replaced with fresh complete McCoy's 5a medium containing 10^{-5} M BrdU, the cultures are incubated in the dark for 21.5 hours, then colchicine is then added at a final concentration of 0.4 µg/ml.

The mitotic cells are harvested by shaking the flasks, and centrifuging the cell suspension. Then, the supernatant is aspirated, and 4 ml of a hypotonic solution of 0.075 M KCl is added to the cells for approximately 7 to 20 minutes at 37°C. The cells are suspended in a fixative of methanol:acetic acid (3:1) three times, and air-dried slides are prepared. The cell density on the slide is monitored using a phase-contrast microscope; at least two slides of appropriate cell density are made from each sample, and the slides are stained by the modified FPG technique (22).

For each test, two cytogeneticists analyze duplicate coded samples of at least three concentrations of the test compound and the positive, negative, and vehicle controls. Each cytogeneticist analyzes 25 cells per sample for the total number of SCEs per cell and for the number of chromosomes per cell. Thus, 50 cells are scored for each analyzable concentration of the test compound and for the positive, negative, and vehicle controls. Occasionally, discrepancies in the evaluations between the two cytogeneticists are noted. Since the cells they evaluate originate from the sample, it is possible that such discrepancies may be a result of differences in the interpretations by the cytogeneticists rather than an effect of the treatment of the cells. Therefore, when this occurs, a third cytogeneticist evaluates 25 different cells from the same coded slides to resolve the discrepancy. The slides evaluated by each cytogeneticist are identified in the tables of results.

Criteria for Interpretation

A valid SCE assay is one in which (a) testing was performed to the maximum concentration expected to yield sufficient M_2 metaphases for analysis, to the limits of solubility, or to the limits of compound availability (a maximum of 5000 μg/ml or 0.5% for liquids is desirable); (b) at least one cytogeneticist observed at least twice the negative control SCE frequencies in a positive control sample; and (c) SCE frequency analyses by at least two cytogeneticists were in general agreement.

Positive. A test compound is considered to have elicited a positive response in the SCE assay if it induces at least twice the baseline SCE frequencies or if at least three concentrations show a progressive increase in SCE frequencies, with at least one value being significant at $P < 0.001$.

Negative. A test compound is considered to have elicited a negative response in the SCE assay if the results obtained by each cytogeneticist are in general agreement and the criteria for a positive response are not met.

Inconclusive. The results of an SCE assay are considered inconclusive if the observations of one cytogeneticist meet the criteria for a positive response but those of the other cytogeneticist do not, or if there is reason to believe that the concentrations of test compound selected for evaluation were inappropriate.

L5178Y $TK^{+/-}$ Mouse Lymphoma Forward Mutation Assay

Background

The L5178Y $TK^{+/-}$ mouse lymphoma mutagenesis assay determines whether a test compound can induce an increased forward mutation frequency at the thymidine kinase (TK) locus. Research on the L5178Y system since its introduction by Clive et al. (30) has established its sensitivity and reproducibility (31-34), and it has been demonstrated that this assay can detect mutagenic activity in several classes of carcinogens that do not show mutagenicity in the Ames assay. Recently, Clive et al. (35) determined that mutagenic potency in the L5178Y system is roughly related linearly to oncogenic potency in 24 of 25 carcinogens tested. In addition, they established that on the basis of colony size, two classes of thymidine kinase-deficient ($TK^{-/-}$) mutants can be observed for most mutagens/carcinogens tested. Large-colony $TK^{-/-}$ mutants are thought to represent single-point or gene mutations affecting the TK locus, whereas small-colony mutants might originate from multilocus (chromosomal) mutations. The relative proportions of these mutants appear to be both mutagen- and dose-dependent.

The major synthetic pathway to thymidine triphosphate (dTTP) for incorporation into DNA includes the enzyme thymidylate synthetase (TS). A secondary pathway for dTTP synthesis exists through the enzyme TK. This "scavenger" pathway uses exogenous thymidine (TdR) or TdR derived from DNA degradation. The selection medium that we use for $TK^{-/-}$ cells contains the thymidine analog trifluorothymidine (TFT). Cells proficient in TK ($TK^{+/+}$ and $TK^{+/-}$) incorporate TFT via the TK pathway; the TFT irreversibly inhibits the TS enzyme and, thus, prevents further dTTP synthesis and cell division. Cells deficient in TK ($TK^{-/-}$) are unable to incorporate TFT and, hence, are able to continue dTTP and DNA synthesis, via the TS pathway, to divide, and to form colonies in the presence of TFT (35). For this assay, SRI uses L5178Y mouse lymphoma cells that are heterozygous for thymidine kinase ($TK^{+/-}$), which were originally supplied by Dr. Clive of Burroughs Wellcome Co., Research Triangle Park, NC.

Method

The cells are cultivated in Fischer's medium for leukemic cells of mice (F_0). F_0 is supplemented with 31 µg/ml penicillin (1650 units/mg), 50 µg/ml streptomycin sulfate, 0.1% Pluronic F68, 0.22 mg/ml sodium pyruvate, and 5% heat-inactivated horse serum to make F_{5p}. F_{5p} is the medium used for exposures in the presence of metabolic activation. F_{10p} contains 10% heat-inactivated horse serum but is otherwise the same formulation as F_{5p}. F_{10p} is the medium used for routine cell cultivation, for exposures in the absence of metabolic activation, and for cell growth during the expression period. Cloning medium differs in that Pluronic F68 is omitted, the horse serum concentration is 20%, and 0.35% Noble agar is added. The selective cloning medium contains 2.5 µg/ml TFT.

Depending on the solubility of the test material, the solvent of choice is water, DMSO, ethanol, or acetone. If more than one solvent can be used, preference is given in the order listed here. If information on the test chemicals' solubility is not available, a preliminary solubility test is performed. Sonication is used to achieve the maximum solubility--or even suspension--of the compounds. Immediately before each assay, an aliquot of chemical is diluted in solvent to form one or more concentrated stock solutions. Stock solution and additional solvent (to form a total volume of 0.1 ml) are added separately to each cell-suspension culture. Concentrations are expressed in µg/ml.

The positive control compounds are ethyl methanesulfonate (500 µg/ml), an alkylating agent that induces mutagenesis without metabolic activation, and 3-methylcholanthrene, which induces mutagenesis only with metabolic activation. An appropriate concentration of 3-methylcholanthrene (between 1 and 15 µg/ml) is selected according to the activity of each metabolic activation preparation. The solvent control samples contain the same concentration of solvent as the treated samples. For assays with metabolic activation, the activation mixture is also added to all controls. The negative control for assays both with and without metabolic activation has only solvent added. Duplicate samples of the positive control, negative control, and solvent controls are used for each test.

The metabolic activation system is prepared identically to that for the SCE system (see above) except that the activation mixture consists of 1 ml of S-9, 2 ml of F_0, and appropriate cofactors (25 mg of NADP and 45 mg of sodium isocitrate). This mixture is added to 76 ml of 8.75×10^5 cells/ml of F_{5p} to form the exposure system in the presence of metabolic activation.

A preliminary concentration range-finding assay of each compound is performed, using 18 concentrations over a 3-log range, both in the absence and in the presence of metabolic activation, and the exposed cell suspensions are treated in the same way as those to be used for the mutagenesis assay. The results of the preliminary assay are employed to determine the cytotoxicity of the test compound and to establish the concentration range to use in the mutagenesis assays. For use in the mutagenesis assays, ten concentrations of the test compound are selected to reflect cell survival in suspension culture ranging from approximately 10 to 95% of the survival rate in the solvent control.

The preliminary concentration range-finding assays and the mutagensis assays are performed similarly. L5178Y $TK^{+/-}$ cells (6×10^6) are exposed to 100 µl of the dilution of a compound in 10 ml of medium in a sterile, 50-ml centrifuge tube. Exposures in the absence of activation are in 10 ml F_{10p}; exposures with metabolic activation are in 7 ml of F_{5p} plus 3 ml of the activation mixture. The centrifuge tube is rotated for 4 hours in a roller drum held in an incubator at 37°C. Then the test chemical is removed by washing the cells twice with 10 ml of F_{10p}, and 6×10^6 cells are resuspended in 15 ml of F_{10p} per 50-ml centrifuge tube, for a final density of 4×10^5 cells/ml. The tubes are rotated in a roller drum for 2 days for expression of any mutations. During the expression period, cell density is maintained between 3 and 15×10^5 cells/ml. Cell growth is monitored daily by adding 1 ml of cells to 9 ml of 0.1% trypsin in saline, incubating the suspension for 10 minutes at 37°C, and then counting the cells (particles) in a Coulter counter. After the expression period, the cells--at appropriate concentrations--are mixed evenly with soft agar cloning medium, and the mixtures are poured into petri dishes. For each sample, 3×10^6 cells are plated in cloning medium supplemented with 1 to 10 µg/ml TFT; 600 cells are plated in nonselective cloning medium to determine viability.

After the cells have grown for 11 days at 37°C in an atmosphere containing 5% CO_2 in air, colonies of cells in each petri dish are counted.

Criteria for Interpretation

The mutation frequency is calculated by dividing the number of mutant cells per milliliter of original suspension culture by the number of viable cells per milliliter of original suspension culture. Therefore, the mutation frequency is the ratio of the number of mutant cells to surviving cells at each concentration of the test chemical. The average mutation frequency of the solvent control samples is subtracted from that of each treated sample so that the results can be expressed as induced mutation frequencies.

The criteria for determining whether the results of an L5178Y mouse lymphoma forward mutation assay are valid are (a) the cloning efficiency of the solvent control is between 60 and 120%, (b) the spontaneous mutation frequency of the solvent control is approximately 100 x 10^{-6} or less, (c) the mutation frequency of the positive control and its survival level are within the range of historical averages, (d) the number of concentration levels tested (e.g., four) is sufficient to yield interpretable data, and (e) the concentrations tested yield cell survivals that are approximately 10 to 95% of the solvent control survival rate.

Positive. The test results generally are considered positive if the mutation frequency of at least one concentration of the test compound is at least twice that of the solvent control and if there is a concentration-related increase in the mutation frequency with increasing concentration of the test compound.

Negative. The results are considered negative if the test is valid but none of the treated samples shows a mutation frequency at least twice that of the solvent control and there is no indication of a concentration-related response for the mutation frequency.

Inconclusive. The results are considered inconclusive if a compound shows limited or no cytotoxicity at the highest testable concentration (e.g., the highest soluble concentration) or if there is a lack of reproducibility in repeat assays that might be due to compound instability or other causes.

Unscheduled DNA Synthesis Assay of WI-38 Cells*

Background

The liquid scintillation counting (LSC) unscheduled DNA synthesis (UDS) assay, using WI-38 cells (human diploid fibroblasts) as target cells, is based on an indirect measurement of DNA damage. UDS is the incorporation of nucleotides into the DNA of cells during repair of damage induced by physical or chemical agents. We use LSC to measure the incorporation of tritiated thymidine (^{3}H-TdR) into the DNA.

UDS occurs in a variety of mammalian (and plant) cell types and has been observed in all phases of the cell cycle (G_0, G_1, G_2, and M) other than S, the normal synthetic phase (36). UDS is not observed during the S-phase because of the much greater incorporation of nucleotides into DNA during scheduled DNA synthesis, which obscures observations of nucleotides being incorporated by UDS.

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*Under the present contract, this assay system was used only for retests of six previously tested compounds.

The WI-38 UDS test system is considered to be an informative *in vitro* assay because a diploid human cell is used to measure genotoxic effects of potentially hazardous materials, and because many mutagenic and carcinogenic agents have been shown to induce UDS in an *in vitro* tissue culture system of cells (36). Thus, the UDS approach is relevant for extrapolations from the *in vitro* test to the normal *in vivo* situation.

The WI-38 cells are a well-characterized continuous cell line that fulfills the criteria for primary screening of hazardous materials (37). The criteria include the following attributes: (a) stable cell line, (b) generation time of approximately 24 hours, (c) mycoplasma-free, and (d) grown readily and reproducibly on commercially available serum and media.

When using the WI-38 cell LSC UDS assay to test potentially hazardous materials, the assay must be supplemented with a metabolic activation system; we routinely use a microsomal preparation from adult male Swiss-Webster mouse liver homogenates. A parallel series of UDS assays in the presence and in the absence of the metabolic activation system is performed for each test compound.

Method

WI-38 cells grown in T-25 tissue culture flasks are used for each UDS assay. Replicate cultures of these cells are initiated in Eagle's Basal Medium containing 10% (v/v) FCS. The cells are grown to confluency and maintained in medium containing 0.5% serum for 5 to 6 days preceding the UDS assay. This produces contact-inhibited cells in synchronous culture in the G_0 phase of the mitotic cycle. To reduce the possibility of incorporation of ^{3}H-TdR by an occasional S-phase cell that might escape the contact-inhibition synchrony and thus obscure measurements of UDS, the cultures are preincubated for 1 hour with 10^{-2} M hydroxyurea (HU) before each assay, and 10^{-2} M HU is added during each subsequent step of the assays.

Immediately before each assay, the test compound is diluted in DMSO to form a series of concentrations that, when diluted into culture medium, yield the appropriate set of test concentrations. When the formula of the compound is known, concentrations are expressed in molar units. The final concentration fo DMSO is maintained at 0.5%, thus minimizing the possibility of a cytotoxic effect in response to the solvent.

For testing with metabolic activation, we use a preparation consisting of the 9000 x *g* supernatant of a liver homogenate (250 mg of liver/ml) from adult male Swiss-Webster mice. To this are added the following cofactors: nicotinamide, 3.05 mg/ml; glucose-6-phosphate, 16.1 mg/ml; $MgCl_2 \cdot 6H_2O$, 5.08 mg/ml; and NADP, 0.765 mg/ml.

The positive controls are 4-nitroquinoline-N-oxide (4NQO), a compound that induces UDS in the absence of metabolic activation, and dimethylnitrosamine (DMN), a compound that induces UDS *in vitro* only with metabolic activation. The negative control is DMSO diluted in culture medium. (Preliminary tests were not conducted, since the results of the earlier studies done under a previous contract were available for use in selecting the concentration ranges for the retests.)

The contact-inhibited WI-38 cells are incubated at 37°C with dilutions of the test compound and with 1 µCi/ml of ^{3}H-TdR (sp act, 6.7 Ci/mmole). For testing in the absence of metabolic activation, the cells are exposed simultaneously to the test compound and to ^{3}H-TdR for 3 hours. For testing with metabolic activation, the cells are incubated with the test compound, ^{3}H-TdR, and the metabolic activation preparation for 1 hour and then with only ^{3}H-TdR in culture medium for an additional 3 hours. (The shorter exposure time for metabolic activation testing prevents cytotoxic effects to the WI-38 cells by the liver homogenate preparation.) To extract DNA from the cells, we use a modification of the PCA-hydrolysis procedure (38); one aliquot of the DNA solution is used to measure the DNA content, after reaction with diphnylamine (39), and a second aliquot is used for scintillation counting measurements of the extent of incorporation of ^{3}H-TdR. Results are expressed as disintegrations per minute (dpm) of incorporated ^{3}H-TdR per unit of DNA and are compared with the rate of incorporation of ^{3}H-TdR into cells exposed to solvent only (negative control).

The above procedure differs somewhat from the procedure used in the previous testing. Previously, the WI-38 cells were incubated for 4 hours with ^{3}H-TdR alone following exposure for 1 hours to the test compound, ^{3}H-TdR, and the metabolic activation preparation. In the retests, the cells were incubated with ^{3}H-TdR for only 3 hours following the metabolic activation exposure step because we had found, in UDS testing of other compounds, that comparable results were obtained with this shorter, more efficient incubation time. Also, in the earlier study, only three concentrations of test compound were tested, and only three replicate samples were used for each concentration of test compound and for the positive and negative controls. The retesting was conducted using five concentrations of test compounds for each assay--to enhance the possibility of detecting any concentration-related increase in UDS--and using six replicate samples for each concentration of test compound and for the positive and negative controls--to facilitate the statistical analysis of the results.

Criteria for Interpretation

Positive. A test material is considered positive if there is a significant concentration-related increase in ^{3}H-TdR incorporation over that of the solvent control.

Negative. A test material is considered negative if no significant concentration-related increase in ^{3}H-TdR incorporation is observed.

Inconclusive. An assay is considered inconclusive if neither of the two above situations is observed.

In Vivo Assays

Mouse Micronucleus Test

Background

The micronucleus test is a rapid, in vivo cytogenetic screen. It is based on the observation that cells with chromosome breaks and/or exchanges often have disturbances in the distribution of chromatin during cell division. After division, the daughter cells contain this displaced chromatin as distinct micronuclei in the cytoplasm (40).

The cell population tested consists of erythroblasts undergoing their final chromosome replication and mitosis before expulsion of the nucleus. Bone marrow or peripheral blood smears made at intervals after treatment yield a population of young, enucleated erythrocytes that are known to have undergone mitosis during the time of treatment. These young erythrocytes are readily identifiable; they stain differently from mature cells and they are easily scored for the presence of micronuclei because they have no nucleus.

Because it indicates in vivo chromosome damage and/or spindle apparatus impairment, the micronucleus test is an important part of the battery of tests used to determine the mutagenic potential of chemicals. Correlated with the results of gene mutation assays and DNA-damage tests, the results of the micronucleus test contribute to the comprehensive evaluation of chemicals.

Method

The method of Schmid (40) is used for administration of test compounds and preparation of bone marrow smears. Two sampling times (48 and 72 hours), in addition to the 30-hour time recommended by Schmid, are evaluated to test adequately compounds that may be slowly metabolized or absorbed.

Water-soluble compounds are administered in Hanks balanced salt solution (HBSS) or water. For compounds not soluble in water, solution in DMSO is attempted. Compounds not soluble in either water or DMSO are suspended in corn oil. Compounds are administered (5 ml of vehicle per kilogram of body weight) in three dilutions: 80%, 40%, and 20% of the LD50. The high-dose-level dilution is prepared using the appropriate amount of compound, and serial dilutions are made to prepare the 40% and 20% levels. Vehicle, positive, and negative (blank) control groups are included with each test. Trimethylphosphate (TMP) is the positive control agent. When more than one compound is tested simultaneously, common controls are used.

If toxicity data are available for a given compound, no preliminary procedures are necessary. If no guidelines are available, a preliminary range-finding test is done to determine appropriate dose levels. This range-finding test can be an informal procedure that allows selection of a toxic dose level that permits the survival of enough animals for the three-day test period.

For the test itself, male Swiss-Webster mice weighing 20 to 30 g are housed four to a cage, with food and water ad libitum, and acclimated for one week. Animals are identified by cage number and ear punch or tail mark. Animals rooms are maintained at a temperature of 72 ± 2°F and on a 12-hour light cycle. The compound to be tested, dissolved in an appropriate vehicle, is administered by gavage or intraperitoneal injection to 30 animals at each dose level tested. Doses are expressed as mg/kg body weight. Vehicle-control animals are given the solvent in the same volume as that received by the treated animals of the same weight, the actual amount (5 or 10 ml/kg) to be determined during the dose range-finding study. Ten positive-control animals receive TMP (1250 mg/kg), administered intraperitoneally in 5 ml/kg of DMSO (the intraperitoneal route is the most effective route for TMP-induced effects). The test compound is weighed and dissolved in the vehicle immediately before dosing. Fresh solutions are made on each of the two days of dosing. Animals are weighed and treated at 0 and 24 hours. Sacrifice with CO_2 and smear preparation are done at 30, 48, and 72 hours after the first dose of test compound.

The eight animals to be sacrificed at each time are selected randomly (using a random-number table) from the animals in each treatment group. To ensure survival of at least eight animals at each dose-time point, each compound-treated group contains more animals than needed for scoring. Extra animals surviving past 72 hours are discarded. Two blood smears are made for each animal by mixing a drop of cardiac blood with a drop of FCS. Three bone marrow smears are made from each animal according to the method of Schmid (40). Slides are stained with buffered Giemsa stain, air-dried, and coverslipped. All slides are coded before they are scored so that the technician is unaware of the identity of the slides being scored. Five hundred polychromatophilic erythrocytes are evaluated for each animal. The number of micronucleated cells is tallied. The ratio of polychromatophilic to mature red cells is calculated from the number of mature cells encountered while scoring 200 polychromatophilic cells.

When scoring is completed, the slides are decoded and the results are compared to a decision table made according to the method of Mackey and MacGregor (41).

Data Manipulation and Presentation

The decision of positive or negative results for any one dose level is dependent on the total number of micronucleated cells observed in the group; therefore, the only data manipulation is the summation of micronucleated cells. The ratio of polychromatic (PCE) to normochromatic (RBC) cells is calculated for each animal. The data of interest--the number of micronucleated cells and the PCE to RBC ratio for each animal, the total number of micronucleated cells in each group, and the mean ratio for each group--are presented in tabular form in the final report.

The method of Mackey and MacGregor (41) is used to determine whether a dose group is positive or negative in the micronucleus test. This method is based on the negative binomial distribution and uses a decision table constructed using the following parameters:

Parameter	Definition	Value in This Study
$\bar{x}_1$	The mean number of micronucleated cells per 500 polychromatophilic erythrocytes (PCE) scored in historical control animals.	0.9
$\bar{x}_2$	The true mean number of micronucleated cells per 1000 PCE needed to declare a compound mutagenic.	3.0
k	The negative binomial constant, estimated from historical control data using a FORTRAN program supplied by Bruce Mackey.	6.9
α	The chance of declaring mutagenic a group whose true mean equals $\bar{x}_1$ (false positive). The α error decreases as the true mean for a group increases.	(from table)
β	The chance of declaring nonmutagenic a group whose true mean equals $\bar{x}_2$ (false negative). The actual β error decreases as the true mean for any particular group decreases.	(from table)

Using these values in the formulae of Mackey and MacGregor, the following decision table has been calculated:

No. of Animals	Negative Decision $\beta <$					No. Decision α & β > 0.10	Positive Decision $\alpha <$				
	0.001	0.005	0.01	0.05	0.10		0.10	0.05	0.01	0.005	0.001
5	< 1	2,3	--	4,5	6	7-10	11	12,13	--	14,15	16
6	< 3	4	5	--	7	8-12	13	14	15	16	18
7	< 4	6	7	8	9	10-14	--	15	17	18	20
8	< 6	7,8	--	10	11	12-15	16	17,18	19	20	21

To use the table, the row for the number of animals in the group is read across until the value for the total number of micronucleated cells found in the group is located. The decision and level of significance of the decision are indicated at the top of the column in which the total is located.

The data generated are considered unacceptable if the value for the positive or the negative control group does not fall within its range in the decision table. If either control group is unacceptable, slides are recoded and evaluated by another observer. If the results remain outside the acceptable range for the group, the study is repeated.

Criteria for Interpretation

Positive. A compound is judged mutagenic if at least two dose-time groups fall within the positive range.

Negative. A compound is judged nonmutagenic if the highest dose given is the maximum tolerated dose and all dose-time groups fall within the negative range. "Maximum tolerated dose" is assumed to have been achieved if there is significant weight loss in the treated groups or if some animals die before scheduled sacrifice.

Inconclusive. Results are considered inconclusive if all dose groups fall in the "no decision" category or if extreme bone marrow depression (mean PCE/RBC ratio < 0.1-0.15) occurs in all groups in a negative test.

Drosophila Sex-Linked Recessive Lethal Assay

Background

The sex-linked recessive lethal (SLRL) test using *Drosophila melanogaster* can detect lethal point mutations and small deletions on the X-chromosome, which constitutes approximately 20% of the *Drosophila* genome. It is one of the most sensitive and reliable short-term mutagenesis assays, and it is the most rapid and economical whole-animal test system for measuring mutation frequencies. The ability of the SLRL test to detect mutagenic effects of pesticides is well documented (42-44).

SRI uses a modification of the *yellow-Bar* test described by Würgler et al. (45) for SLRL determination. In this system, *yellow-Bar* males that carry *BAR* (*B*) and *yellow* (*y*) on the X-chromosome and two minute secondary translocations of the X-chromosome, bearing the wild-type allele of *y* (*y+*), one on each arm of the Y-chromosome, are exposed to the test chemical. The exposed males are crossed singly to females carrying the *Inscy* X-chromosome in homozygous condition. This doubly inverted X is marked with *y* and *scute* (*sc*). The F_1 female progeny, each of which represents one treated X-chromosome, are mated to their F_1 brothers. Each F_1 female is then placed in a separate vial for egg-laying. Lethality is scored by the absence of the treated male phenotype in the F_2 generation.

The *yellow-Bar* test has the following advantages: (a) XXY females can be excluded from the F_1 mating population quickly and easily, (b) reciprocal translocations can be detected using the same stocks, and (c) sex chromosome loss and nondisjunction can be detected. Two strains of *Drosophila* are used.

Yellow-Bar stock: $C(1)DX, y\ f/y\ b/y^+\ Y\ y^+$. The females of this stock have an attached X-chromosome marked with the recessive mutations *y* and *forked* (*f*). The X-chromosome possessed by the male carries *y* and the dominant mutation *B*. The Y-chromosome, present in both males and females, carries two very small translocated pieces of the X-chromosome, one on each arm, bearing y^+. Therefore, this stock is phenotypically wild-type in body color. To initiate the SLRL assay, males from this stock are exposed to the test chemical. Lindsley and Grell (46) describe this stock and the following chromosomes and mutations more completely. This stock was produced at SRI by crossing two stocks obtained from the stock centers at Bowling Green State University, Bowling Green, Ohio, and Madrid, Spain.

"Oster" stock: In(1)sc^{S1L} sc^{8R} +dl-49,y/y$^+$ Y; bw; st p^p. The *Inscy* X-chromosome contains a double inversion to render cross-over gametes lethal, and it is marked with the visible recessive mutations *sc* and *y*. The stock is homozygous for the following recessive eye color mutations: brown (bw) on the second chromosome, and *pink peach* (*p^p*) and *scarlet* (*st*) on the third chromosome. This stock supplies the females for crossing with the treated males in the parental (P_1) mating. The *y$^+$* on the Y-chromosome prevents the accidental use of an XXY female, because any fly with a Y-chromosome has a wild-type rather than yellow body color. This stock was produced at SRI by adding the *y$^+$ y* to an "Oster" stock obtained from Bowling Green State University.

Method

The compound to be tested is dissolved in an appropriate solvent. The choice of solvent is based on physical data (including solubility). When possible, this solvent is an aqueous solution of 5% sucrose. If this is not possible, the chemical is dissolved in ethanol, DMSO, or another appropriate solvent (e.g., clarified butter or peanut oil).

The test compound is diluted in the same solvent in which it was dissolved. If a solvent other than water is used, the compound is further diluted in an aqueous 5% sucrose solution to give a final concentration of 5% sucrose and 1% to 2% solvent, concentrations that have no harmful effects on *Drosophila*. Concentrations of test chemical are expressed in percent.

The vehicle control consists of the solvent used to dissolve the chemical, diluted in an aqueous 5% sucrose solution.

The positive control is 0.01% ethyl methanesulfonate (EMS) in aqueous 5% sucrose solution. At this dosage, over a 3-day exposure, EMS produces 9.6% to 36.8% SLRLs in our laboratory, with no apparent reduction in viability or fertility.

Preliminary range-finding tests are used to determine the test material's palatability, toxicity (including determination of an LD50) and effect on fertility. The compound is tested at eight concentrations, the maximum concentration being 1 mg/ml or less, depending on solubility, and the minimum being approximately 0.01 µg/ml.

Test compounds are administered to adult male *Drosophila* by feeding. Although we cannot control the individual dose when it is administered by feeding, we can determine whether the flies are ingesting the chemical. To determine the palatability of the concentrations of chemicals being tested, USDA-certified green food coloring is added to each concentration of the chemical dissolved or suspended in 5% sucrose solution. If the flies eat the solution, the green dye in their guts can be seen through their cuticles. Since this is the only food and moisture available to the flies for 3 days, they must eat or die of starvation/dehydration. If they die, the green food coloring allows us to determine qualitatively whether the mortality was caused by toxicity of the chemical or refusal to eat.

Male $\underline{y\ B/y^+\ Y\ y^+}$ Drosophila, aged 3 to 4 days with their sisters before separation into groups of 10, are exposed to the compound being tested and to the negative control in cotton-plugged, standard glass, 25 x 94 mm shell vials lined on the bottom and inside with Gelman glass-fiber filter (type AE) saturated with the test solution. Each day of the 3-day exposure period, flies are shaken into an identical vial containing freshly prepared test solution. During these transfers, the flies are shaken into empty vials just long enough to observe whether their guts are green. After the third day of exposure, lethality and palatability for each dose level are recorded. The surviving males are mated individually to three virgin females of the "Oster" stock. Seven days after initiation of this cross, fertility is determined, by counting the number of vials containing viable third instar larvae or pupae at each concentration. The concentration expected to cause 50% toxicity or sterility in the exposed males, whichever is lower, is used as the test concentration. Offspring are observed again at 14 days after the initiation of the crosses to ensure that eclosion occurs, to record any effects of delayed development caused by the compound, and to reaffirm that the chosen doses are correct. If no concentration of the compound causes this degree of lethality or sterility, the compound is tested at 1 mg/ml.

For the SLRL test itself, newly eclosed males of the genotype $\underline{y\ B/y^+\ Y\ y^+}$ are aged 3 to 4 days with females before being separated into groups of 10 for exposure. This aging procedure is intended to avoid spuriously high mutation frequencies in mature sperm that may be produced by Drosophila males just after eclosion (45). On the third or fourth day after eclosion, these males are exposed to the test compound (at a dose level chosen on the basis of preliminary testing) or to the negative, vehicle, or positive control mixture by a 3-day feeding regimen. The feeding protocol is the same as that described earlier except that green food coloring is omitted from the feeding solutions.

A numerical coding system is employed after exposure and before P_1 mating, and is recorded in a code book. The system assigns a unique number to each male; in this way, the mating and scoring of the experiment cannot be biased by knowledge of how that male was treated. After the scoring is completed, the code is revealed and each treatment is analyzed separately.

Appropriate numbers of males from each treatment group are mated individually to two or three virgin "Oster" females (aged approximately 3 to 5 days for maximum mating receptivity) to ensure the desired sample sizes. After three days with the Brood 1 females, each male is introduced to a new group of three virgin "Oster" females for Brood 2. A total of three broods of 3 to 5 days' duration is tested in this way to sample sperm exposed at various times during spermatogenesis (47). Broods 1 (Days 1 through 3) and 2 (Days 4 through 6) are designed to sample sperm that were in postmeiotic stages (spermatids and spermatozoa) at the termination of treatment. Brood 3 (Days 7 through 10) tests sperm that were undergoing meiosis as spermatocytes at the termination of treatment.

Parents are discarded from each vial at the time of pupation of the F_1 progeny. Approximately 1 week after the F_1 progeny in each brood begin to emerge, they are anesthetized with CO_2 and examined. White-eyed F_1 progeny indicate that a nonvirgin "Oster" female was used inadvertently (since the "Oster" stock produces white-eyed flies due to recessive mutations on the second and third chromosomes), and such flies are discarded. F_1 females that are wild type in body color are exceptional XXY females and are not used to make F_1 crosses. For each treated male, F_1 females of the yellow-Bar phenotype are mated individually with one or more male sibs (or "Oster" stock males).

Since 5 to 10 F_2 females derived from each treated male are tested per brood for four broods, approximately 20 to 40 X-chromosomes from each treated male are tested. Data are recorded so that each F_1 female tested can be traced back to the treated male that produced it. This permits identification of "clusters" of mutations (see below).

The F_2 cultures (from which F_1 parents need not be discarded) are kept for 12 to 15 days after initiation of the brood. Cultures are examined through the glass vial at low power under a dissecting microscope after anesthetization with CO_2. If at least two Bar-eyed males are present, the culture is scored as nonlethal and discarded. If a culture appears to be missing Bar-eyed males, the flies are anesthetized and examined more closely. If there are at least 20 male and female progeny total (discounting F_1 parents) and no Bar-eyed males, the culture is retested for confirmation. If less than 20 progeny are present with one or no Bar-eyed males or if the ratio of Bar-eyed males to Bar-eyed females is low, the culture is retested. Three F_2 females of the yellow-Bar phenotype are mated with their F_2 male sibs (or "Oster" males).

The F_3 progeny are examined and analyzed as follows. If there are more than 20 male and female F_3 progeny total and no Bar-eyed males, the culture is scored as lethal. If some F_2 females test lethal and some F_2 females test nonlethal, the gonads of the F_2 females were mosaic for an SLRL, and this is scored as a lethal. If less than 20 progeny emerge from all three F_3 cultures, this chromosome is eliminated from the final calculations.

Each compound is tested once by feeding, using the SLRL protocol. If the induced mutation frequency is high enough that the increase above the negative control mutation frequency can be determined to be significant with 95% confidence, the compound is not retested by the SLRL procedure. If a significant increase in the mutation rate cannot be demonstrated after one test, the compound is retested once or twice, until sufficient chromosomes have been tested to permit detection of a 0.2% increase over the negative control mutation frequency with 95% confidence, based on the tables of Würgler et al. (48).

On completion of the scoring, the experiment is decoded and the tabulated results are statistically analyzed. The SLRL mutation frequency is determined by dividing the number of F_1 females possessing recessive lethal mutations of the treated X-chromosome by the total number of F_1 females successfully tested. Brood and overall frequencies are determined for each experimental and control group.

Clusters of mutations detected in F_2 progeny of the same treated F_1 males (defined as one family) are considered in determining mutation frequencies. If the family sizes do not vary greatly, we apply Engels' formula (49) for cluster correction, which results in reduced variance over the uncorrected analysis. Otherwise, following the recommendation of R. Valencia (personal communication), clusters with at least four mutations are considered to have arisen spontaneously during premeiotic stages and are eliminated; smaller clusters are noted, but are treated as individual events.

To determine whether the mutation frequency determined for the treated group is significantly greater than that observed in the negative control, the significance test developed by Kastenbaum and Bowman (50) is used. This test is based on a Poisson distribution, which fits *Drosophila* mutagenicity data better than a binomial distribution, especially when the total number of mutations counted is less than 200.

Criteria for Interpretation

Positive. A compound that induces at least a 0.2% increase over the background SLRL mutation rate (tested with a sample size adequate to permit detection of this increase with 95% confidence) is identified as having elicited a positive response.

Negative. A compound that is tested with an adequate sample size but that does not induce a 0.2% increase over the background at either dose level is identified as having tested negative.

Inconclusive. A compound that is tested in insufficient numbers to permit detection of a 0.2% increase with 95% confidence is identified as being inconclusively tested for mutagenicity.

RESULTS AND DISCUSSION

General

Table 1 lists the 79 chemicals tested and the common or trade name, manufacturer, purity, identification and supplier for each chemical.

For those eleven chemicals where more than one supplier provided material, Table 2 relates the specific tests to the specific material.

Table 3 summarizes the test results for each of the 79 chemicals evaluated under this contract, the previous contract (51-54), or both. All cases where a retest was done are identified by footnotes, as are cases where reexamination of previous data resulted in a change in interpretation. The *S. typhimurium* plate incorporation results are scored as positive when the results of any of the individual strains are positive. The totals indicate the numbers of tests conducted, excluding inconclusive tests, and the numbers of tests with positive results. For computing the totals, tests with and without activation are scored as positive when either result is positive. Each of the three parameters in the *S. typhimurium* D7 system and each of the four strain pairs in the relative toxicity assays is counted as a separate test.

Table 4 summarizes the extent of concordance between the test results for all 79 chemicals, both as fractions and as percentages. Inconclusive results were excluded. For tests including metabolic activation, the results were counted as positive when either the with-activation or the without-activation results were positive. Caution should be used in considering those pairings where the number of conclusive tests was low (e.g., *Drosophila* SLRL results). On an all-test basis, the median concordance among the 83 comparisons was 67%. For the microbial tests only, the median concordance among the 28 comparisons was 75%, and for the 10 mammalian comparisons only, it was 54%.

The remainder of this report consists of the findings for each of the 41 pesticides and the 10 industrial chemicals tested under the present contract. Within these two categories, the chemicals are in alphabetical order. For each chemical, the test results are in the same order as in Tables 1-3. The descriptive text is based on the individual reports submitted during the course of this project, and the data tables are exact copies of those in the individual reports.

TABLE 1. SEVENTY-NINE CHEMICALS EVALUATED

Common Name	Alternate Name	Manufacturer	Identifying Number	Purity (%)	Supplier
PESTICIDES					
Acephate	Orthene	Chevron	SX-734	93.5	EPA/Battelle
	Orthene	Chevron	SX-762	93.5	Battelle
Aspon	Tetra-n-propyl dithionopyrophosphate	Stauffer	3714-35	93.0	Battelle
Allethrin	Bioallethrin	S. B. Penick	7806-RS	93	EPA
Azinphos-Methyl	Guthion	Chemagro	411-0229	Tech	EPA/Battelle
	Guthion	Chemagro	411-0229	Tech	Battelle
Benomyl	Benlate	DuPont		99	EPA
	Benlate		5-76	99	Chem Service Inc.
Biphenyl	Diphenyl	UC Riverside			EPA
Dichloran*	Botran			95	EPA
Bromacil	Hyvar	DuPont	T80619/40	95.9	Battelle
sec Butylamine 2AB					EPA
sec Butylamine 2AB·H_3PO_4	Freshgard 604				EPA
Cacodylic Acid	Phytar 138	Ansul		65.6	EPA/Battelle
	Phytar 138	Ansul		65.6	Battelle
Captan	Orthocide 406	Chevron	SX-640	Tech	EPA/Battelle
	Orthocide 406	Chevron	SX-640	Tech	Battelle
Carbofuran	Furadan	Niagara	C-4717-54A	Tech	Battelle
Chlordimeform	Chlorphenamidine	Ciba-Geigy			EPA
Chlorpyrifos	Dursban	Dow	MH-11114-1	98.9	Battelle
Creosote P1					EPA
Creosote P2					EPA
Crotoxyphos	Ciodrin	Shell	11-PCC-32	85	Battelle
2,4-D Acid	2,4-Dichlorophenoxyacetic acid	Rhodia	610123	98.6	EPA
2,4-DB Acid	Embutox	Rhodia	610123	98.9	EPA
Demeton	Systox	Chemagro		85	EPA/Battelle
	Systox	Chemagro		85	Battelle
Diallate	Avadex	Monsanto	DSAL09025	45.7	Grand Forks Seed Co.
	Avadex	Monsanto		Tech	EPA
Diazinon	Neocidol, Basudin	Ciba-Geigy	FL-741305	Tech	Battelle
Dicamba	Banvel, Mediben	Velsicol		Tech	Battelle
m-Dichlorobenzene	1,3-Dichlorobenzene	Aldrich	MC-011377	98	EPA

TABLE 1. SEVENTY-NINE CHEMICALS EVALUATED (continued)

Common Name	Alternate Name	Manufacturer	Identifying Number	Purity (%)	Supplier
o-Dichlorobenzene	1,2-Dichlorobenzene	Aldrich	MB-100577	98	EPA
p-Dichlorobenzene	1,4-Dichlorobenzene	Aldrich	LC-120277	97	EPA
Dinoseb	Premerge	Dow	NH200554	97.7	Battelle
DL-cis/trans Chrysanthemic acid		S. B. Penick	8451-RW	93% (36.9% cis/63% trans)	EPA
Disulfoton	Di-Syston	Chemagro	5-08-5110	95	EPA
	Di-Syston	Chemagro	5-08-5110	95	Battelle
DSMA	Ansar-8100	Ansul		80.1	Battelle
Endrin	Exp. Insecticide 269	J. Hyman		99.5	EPA
Ethion	Nialate	FMC	3381-5	Tech	EPA
Ethyl chrysanthemate		Stauffer	5272		EPA
Fensulfothion	Dasanit, Terracur-P	Chemagro	5-10-0004	Tech	Battelle
Fenthion	Baytex	Chemagro	4-15-2026	96	Battelle
Folpet	Phaltan	Chevron	3660/403	Tech	EPA/Battelle
	Phaltan	Chevron	SX 579	Tech	Battelle
Fonofos	Dyfonate	Stauffer	CBA0804	96.9	Battelle
Formetanate Hydrochloride	Carzol SP	Nor Am	982-54 2B	92	Nor Am
Malathion	Cythion; malathon	American Cyanamid	40216006.300	Tech	Battelle
Mancozeb	Dithane M-45	Rohm and Haas			EPA
	Manzate 200	DuPont			EPA
Maneb	Dithane M-22	Rohm and Haas			EPA
	Manzate D	DuPont			EPA
Methomyl	Lannate	DuPont	6602-82	99	Battelle
Methoxychlor	Marlate	DuPont	6543-108	Tech	Battelle
Monocrotophos	Azodrin	Shell	9-SCL-77	58.4	EPA/Battelle
	Azodrin-5	Shell	9-SCL-77	55	Shell
Monuron	Telvar	DuPont	T-40817-20	97	EPA/Battelle
	Telvar	DuPont	T-40817-20	97	Battelle
MSMA	Ansar 170	Ansul	Ansar 170 H.C.	58.4	Battelle
NRDC-149		ICI-America	EX-P14, PP 383 2E	90.5% (37.2% cis/53.6% trans)	EPA
Parathion	Niran	Monsanto	AD-1236	99	Battelle
Parathion-Methyl	Methyl Niran	Monsanto	AD-0659	80	EPA/Battelle
	Methyl Niran	Monsanto	AD-0659	80	Battelle
PCNB	Quintozene	Olin Mathison		99	Battelle

TABLE 1. SEVENTY-NINE CHEMICALS EVALUATED (concluded)

Common Name	Alternate Name	Manufacturer	Identifying Number	Purity (%)	Supplier
Pentachlorophenol	Dowicide PC-7	Dow	MMD6168	88	EPA
Permethrin	NRDC-143	ICI-America	BX-61223	90.4% (38.3% cis/52.3% trans)	EPA
Phorate	Thimet	American Cyanamid	MC-85	85	Battelle
Polyram	Metiram	FMC	C7606-29-B	Tech	EPA
Propanil	Stam Tech	Rohm and Haas	6-2402	88	EPA
Resmethrin	SBP-1382	S. B. Penick	3521-LTV-3	90	EPA
Rotenone	Protex	Fairfield Amer. Corp.	FMB-77-2-262	41.59	EPA/Battelle
Siduron	Tuperson	DuPont	I-70715-C	96.7	Battelle
Simazine	Primatol	Ciba-Geigy	FL-740846	97.7	EPA/Battelle
	Primatol	Ciba-Geigy	FL-740846	97.7	Battelle
Sumithrin	Fenitrothion			94.4	EPA
2,4,5-T	Dow 2,4,5-T Acid	Dow		98.7	EPA
Triallate	Far-go	Monsanto	TTBM08017	46.3	Grand Forks Seed Co.
	Far-go	Monsanto		Tech	EPA
Trichlorfon	Dylox, Dipterex	Chemagro	5-00-7003	Tech	EPA/Battelle
	Dylox, Dipterex	Chemagro	5-00-7003	Tech	Battelle
Trifluralin	Treflan	Eli Lilly	X-26290	97.7	Battelle
Sulfallate*	Vegadex	Monsanto		Tech	EPA
Zineb	Dithane Z-78	FMC	337	Tech	EPA
INDUSTRIAL CHEMICALS					
Acetonitrile		Aldrich	TC112987	99	EPA
Chlorobenzene	Chlorobenzene d_5	Aldrich	102087 PC	99	EPA
Coal Tar - Flaked Pitch		Koppers			Koppers
Coal Tar - Bitumastic A	Bitumastic #300-M, Component A	Koppers	178C1		Koppers
Coal Tar - Bitumastic B	Bitumastic #300-M, Component B	Koppers	162C		Koppers
Coal Tar - Bitumastic A + B					
Ethanol	Ethyl Alcohol	Aldrich		99.5	EPA
Methanol	Methyl Alcohol	Aldrich		99.5	EPA
Toluene	Toluene Nanograde	Mallinckrodt	8092J2	99.9	Mallinckrodt
Vinyl Fluoride		Linde		99.9	Linde

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*Alternate name used in this report.

TABLE 2. DISTRIBUTION AMONG TEST SYSTEMS FOR CHEMICALS FROM TWO SOURCES

Material	Source	S. typh. Pl. Inc.	E. coli WP-2	S. cere. D3	S. cere. D7	E. coli/ B. subt.	S. typh. Rel. Tox.	SCE CHO	L5178Y Lymph	UDS WI-38	Mouse Micro	Dros. SLRL	Mouse DL
Acephate	EPA/Battelle Battelle	●	●	●	●	●	●	●	●	●	●		
Azinphos-Methyl	EPA/Battelle Battelle	●	●	● ●		●	●	●	●	●	●		●
Benomyl	EPA Chem Services, Inc.							●	● ●		●		
Captan	EPA/Battelle Battelle	●	●	●		●	●		●	●			●
Diallate	Grand Forks Seed Co. EPA	● ●	●	●	●				●			●	
Disulfoton	EPA Battelle	●	●	●	●	●	●	●	●	●	●		
Monocrotophos	EPA Battelle Shell	●	●	●	●	●	●	●	●	●	●		●
Monuron	EPA/Battelle EPA	●	●	●	●	●		●	●	●	●		
Parathion-Methyl	EPA/Battelle Battelle	●	●	●	●	●	●	●	●	●			●
Simazine	EPA/Battelle Battelle	●	●	●	●	●	●	●	●	●	●		
Triallate	Grand Forks Seed Co. EPA	● ●	●	●	●				●			●	
Trichlorfon	EPA/Battelle Battelle	●	●	●	●	●	●	●	●	●	●		

TABLE 3. SUMMARY DATA FOR 79 CHEMICALS

	S.typh. Pl. Inc.	E. coli WP-2	S. cere. D3	S. cere D7 MCO	S. cere D7 MGC	S. cere D7 RM	Relative Toxicity E. coli PolA	Relative Toxicity B. subt Rec	Relative Toxicity S. typh. uvrB	Relative Toxicity S. typh. Rec	SCE CHO	L5178Y Lymph	UDS WI-38	Mouse Micro	Dros SLRL	Mouse DL	No. ± Tests	No. Pos.
PESTICIDES																		
Acephate	+ +[a]	- -	+ +	+ +	+ +	- +	?[b]	?	-	-	+ +	+ +	+ -	-			12	8
Allethrin	- +	- -	- -				?	-					- -				5	1
Aspon	- -	- -	- -				?	?					- -				4	0
Azinphos-Methyl	-[c] -[c]	- -	+[c] -[d]	- -	- -	- -	?	?	-	-	? -	- +	-[c] -[d]	-		-	13	2
Benomyl											+ +	+ +		+			3	3
Biphenyl	- -	- -	- -				?	?					- -				4	0
Botran[e]	- -	- -	- -				-	-									5	0
Bromacil	- -	- -	- -	- -	- -	- -	-	-	-	-	- -	± +	- -	-	+	-	15	1
sec Butylamine 2AB	- -	- -	- -				+	-									5	1
sec Butylamine 2AB H_3PO_4	- -	- -	- -				-	-									5	0
Cacodylic Acid	- -	- -	+[c] +[c]	+ +	+ +	+ +	?	?	-	-	- -	+ +	- -	+			12	6
Captan	+[c] +[c]	+ +	+[c] +[c]				+	+	+	+		+ +	-[c] -[d]			-	10	8
Carbofuran	- -	- -	- -				?	?					- -				4	0
Chlordimeform	- -	- -	- -				?	-					- -				5	0
Chlorpyrifos	-[c] -[c]	- -	- -				+	+	+	+			- -				8	4
Creosote P1	- +	- -	- -				-	-				? +					6	2
Creosote P2	- +	- -	- -				-	-				? +					6	2
Crotoxyphos	- -	- -	+ +	- -	- -	- -	?	?	-	-	- -	+ +	- -	-			12	2
2,4-D Acid	- -	- -	- -				-[d]	+[c]	-	-			- -				8	1
2,4-DB Acid	- -	- -	- -				+	-[c]	-	-			- -				8	1
Demeton	+ +	+ +	+ +	+ +	+ +	+ +	-[d]	+	-	-	+ +	+ -	+ +	-			14	10
Diallate	-[c] +[c]	- -	- +	- -	- -	- -						+ +			+		8	4
Diazinon	- -	- -	- -				?	?					- -				4	0
Dicamba	- -	- -	- -				+	+	-	-			- -				8	2
m-Dichlorobenzene	- -	- -	+ +				+	-									5	2

TABLE 3. SUMMARY DATA FOR 79 CHEMICALS (continued)

	S.typh. Pl. Inc.	E. coli WP-2	S. cere. D3	S. cere D7			Relative Toxicity				SCE CHO	L5178Y Lymph	UDS WI-38	Mouse Micro	Dros SLRL	Mouse DL	No. ± Tests	No. Pos.
				MCO	MGC	RM	E. coli PolA	B. subt Rec	S. typh. uvrB	S. typh. Rec								
o-Dichlorobenzene	- -	- -	- -				+	-									5	1
p-Dichlorobenzene	- -	- -	- -				-	-									5	0
Dinoseb	- -	- -	_c_c				+	+	+	+			- -				8	4
Disulfoton	- -	- -	- -	- -	- -	- -	?	?	-	-	- +	+ -	+ -	-			12	3
DL-cis/trans Chrysanthemic Acid	- -	- -	- -				-	+					- -				6	1
DSMA	_c_c	- -	_c_c				-	-					- -				6	0
Endrin	- -	- -	- -				-	-					- -				6	0
Ethion	- -	- -	- -				-	-					- -				6	0
Ethylchrysanthemate	- -	- -	+ +				-	-					- -				6	1
Fensulfothion	- -	- -	- -				?	?					- -				4	0
Fenthion	- -	- -	- -				?	?					- -				4	0
Folpet	+c+c	+ +	+c+c				+	+	+	+		+ +	_c_d			-	10	8
Fonofos	- -	- -	- -				?	?					- -				4	0
Formetanate Hydrochloride	- -	- -	- -				-	-									5	0
Malathion	_c_c	- -	- -				-	-					- -			-	6	0
Mancozeb (Dithane M-45)	- -	- -	+ +				-	-					- -				6	1
Mancozeb (Manzate 200)	- -	- -	+ +				-	-					+ -				6	2
Maneb (Dithane M-22)	- -	- -	+ -				-	-					- -				7	1
Maneb (Manzate D)	- -	- -	+ +				-	-					+ -				6	2
Methomyl	- -	- -	_c_c				-	-					- -				6	0
Methoxychlor	- -	- -	- -				?	?					- -				4	0
Monocrotophos	+f+f	- -	+c+c	+ +	+ +	+ +	?	?	-	+	+ +	+ +	_d+c	-		-	13	9
Monuron	- -	- -	- -	- -	- -	- -	-	-			- +	+ +	_c_d	+			12	3
MSMA	_c_c	- -	- -				-	-					- -				6	0
NRDC 149	- -	- -	- -				?	?					- -				4	0

TABLE 3. SUMMARY DATA FOR 79 CHEMICALS (continued)

	S.typh. Pl. Inc.	E. coli WP-2	S. cere. D3	S. cere D7			Relative Toxicity				SCE	L5178Y	UDS	Mouse	Dros	Mouse	No. ±	No.
				MCO	MGC	RM	E. coli PolA	B. subt Rec	S. typh. uvrB	S. typh. Rec	CHO	Lymph	WI-38	Micro	SLRL	DL	Tests	Pos.
Parathion	-^{c} -^{c}	- -	- -				-	-					-^{d} -^{c}			-	7	0
Parathion-Methyl	- -	- -	+ +	- -	- -	- -	?	?	-	-	- +	+ +	- -			-	12	3
PCNB	- -	- -	- -				?	?					- -			-	5	0
Pentachlorophenol	- -	- -	+ +				-	+									5	2
Permethrin	- -	- -	- -				?	?					- -				4	0
Phorate	-^{c} -^{c}	- -	- -				-	-					- -			-	7	0
Polyram	- -	- -	- -				-	-					- -				6	0
Propanil	- -	- -	- -				-^{d}	+	+	-			- -				8	2
Resmethrin	- -	- -	- -				?	?					- -				4	0
Rotenone														-			1	0
Siduron	- -	- -	- -				?	?					- -				4	0
Simazine	-^{c} -^{c}	- -	- -	- -	- -	- -	?	?	-	-	- -	? +	- -	-			12	1
Sumithrin	- -	- -	- -				?	?					- -				4	0
2,4,5-T	- -	- -	- -				-	+									5	1
Triallate	- +	- -	- +	- -	- -	- -						+ +			-		8	3
Trichlorfon	+^{c}+^{c}	+ +	+ +	+ +	+ +	+ +	?	?	+	+	+ +	+ +	+ -	-			12	11
Trifluralin	-^{c} -^{c}	-^{c} -^{c}	- -				-	-					- -				6	0
Vegadex^g	- +	- -	- -				-	-									5	1
Zineb	- -	- -	+ +				-	-					- -					
INDUSTRIAL CHEMICALS																		
Acetonitrile	- -	- -	- -				-	-									5	0
Chlorobenzene	- -	- -	- +				-	-									5	1
Coal Tar - Flaked Pitch	+ +	- +	- -				-	-									5	2
Coal Tar - Bitumastic A	+ +	- +	+ -				-	-									5	3
Coal Tar - Bitumastic B	+ +	- +	- +				-	-									5	3
Coal Tar - Bitumastic A + B	+ +	- +	+ +				-	-									5	3

TABLE 3. SUMMARY DATA FOR 79 CHEMICALS (concluded)

	S.typh.	E. coli	S. cere.	S. cere D7			Relative Toxicity				SCE	L5178Y	UDS	Mouse	Dros	Mouse	No. ±	No.
							E. coli	B. subt	S. typh.									
	Pi. Inc.	WP-2	D3	MCO	MGC	RM	PolA	Rec	uvrB	Rec	CHO	Lymph	WI-38	Micro	SLRL	DL	Tests	Pos.
Ethanol	- -	- -	- -				-	-									5	0
Methanol	- -	- -	- -				-	-									5	0
Toluene	- -	- -		- -	- -	- -	- -			- -	- -			-			9	0
Vinyl Fluoride	- -	- -	- -														3	0
No. ± Tests	77	77	76	15	15	15	49	51	19	20	14	19	53	14	2	10	526	
No. Positive	16	8	23	5	5	5	9	11	6	6	8	19	7	3	1	0		132

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[a] Positive response, +; negative response, –; inconclusive response, ?; two symbols in one column indicates without activation, with activation.

[b] For relative toxicity tests, results marked inconclusive were previously reported as negative, but reexamination of data using more stringent criteria indicates results should have been reported as inconclusive (see text).

[c] Retested with same results as previously reported.

[d] Previous results reported as positive, but retest and/or reexamination of data indicates resultr are negative.

[e] Botran used as chemical name in this report - common name is Dicloran.

[f] Previous results reported as negative, but retest and/or reexamination of data indicates results are positive.

[g] Vegadex used as chemical name in this report - common name is Sulfallate.

TABLE 4. CONCORDANCE BETWEEN TEST RESULTS

		Fraction												
		S. typh. Pl. Inc.	E. coli WP-2	S. cere. D3	S. cere. D7	E. coli Pol A	B. subt. Rec	S. typh. uvrB	S. typh. Rec	SCE CHO	L5178Y Lymph	UDS WI-38	Mouse Micro	Dros. SLRL	Mouse DL
Percent	S. typh. Pl. Inc.		69/77	59/76	11/15	34/49	34/50	13/19	16/20	10/13	10/18	47/53	6/12	1/2	7/10
	E. coli WP-2	90		58/76	12/15	37/49	38/50	15/19	15/20	8/13	4/18	46/53	8/12	1/2	8/10
	S. cere. D3	78	76		9/14	30/48	31/50	9/19	11/19	7/12	12/18	42/53	4/11	1/2	5/10
	S. cere. D7	73	80	64		3/4	3/3	7/11	9/12	9/13	5/14	10/12	7/12	1/2	3/4
	E. coli PolA	69	76	63	75		38/48	7/9	9/10	2/4	2/7	18/27	3/4	—	4/6
	B. subt. Rec	68	76	62	100	79		7/10	6/10	2/3	3/7	20/30	1/3	—	4/6
	S. typh. uvrB	68	79	47	64	78	70		17/19	6/11	2/12	10/19	8/10	—	4/6
	S. typh. Rec	80	75	58	75	90	60	89		8/12	3/12	12/19	8/11	—	3/6
	SCE CHO	77	62	58	69	50	67	55	67		8/13	10/12	7/12	—	2/4
	L5178Y Lymph	56	22	67	36	29	43	17	25	62		5/14	3/12	1/2	0/6
	UDS WI-38	89	87	79	83	67	67	53	63	83	36		4/11	—	9/10
	Mouse Micro	50	67	36	58	75	33	80	73	58	25	36		—	3/3
	Dros. SLRL	50	50	50	50	—	—	—	—	—	50	—	—		—
	Mouse DL	70	80	50	75	67	67	67	50	50	0	90	100	—	

Pesticides

Acephate

S. typhimurium Plate Incorporation. Acephate was previously tested and was found to be weakly mutagenic with and without activation in strain TA100 and negative in strains TA1535, TA1537, and TA1538 (52).

E. coli WP-2. This compound was previously tested and found to be negative in this system (52).

S. cerevisiae D3. Acephate was previously tested and found to be recombinogenic with and without activation (52).

S. cerevisiae D7 (Tables 5 and 6) This pesticide was first tested over a concentration range of 1.0 to 5.0% and retested at concentrations of 3.0 to 5.0%. It induced mitotic crossing-over and gene conversion in both the presence and the absence of metabolic activation. Reverse mutation was observed only in the presence of metabolic activation.

E. coli/B. subtilis Relative Toxicity. This compound was previously tested and reported to be negative in both species (52). Reevaluation of these results indicates that they are more appropriately regarded as inconclusive, since no discernible zones of inhibition were observed in either strain of either species.

S. typhimurium Relative Toxicity (Table 7). Clear zones of inhibition were apparent for both strain pairs exposed to acephate, but no differential toxicity was apparent between the strains. Thus, acephate was negative in this test.

SCE in CHO Cells (Tables 8-11). Acephate was dissolved in 95% ethanol at a concentration of 500 mg/ml. At 5000 µg/ml, acephate was cytotoxic to CHO cells; at a concentration of 1000 µg/ml, the cultures yielded primarily M_1 metaphases. At 200 µg/ml and less, M_2 metaphases were produced (Table 8). Table 9 presents the results of the cytotoxicity test of acephate in CHO cell cultures with metabolic activation. At a concentration of 1000 µg/ml, M_1 and M_2 metaphases were produced in approximately equal numbers. At other concentrations, primarily M_2 metaphases were produced. Since the CHO cells exposed to acephate at 5000 µg/ml grew normally, we interpret the slight cell cycle retardation observed in the culture exposed to 1000 µg/ml to represent an artifact rather than a toxic effect of acephate.

Table 10 presents the SCE frequencies in CHO cells exposed to acephate without metabolic activation. A one-way analysis of variance was conducted on SCE frequencies of the five concentrations of acephate and of the negative controls. The variance between treatment groups was signficantly greater than the variance within treatment groups. The response appeared to be concentration-related. The maximum effect (approximately 3.2 to 5.0 times the negative control SCE frequencies) was seen at 2000 µg/ml. Table 11 presents the SCE frequencies in CHO cells exposed to acephate with metabolic activation. The variance between treatment groups was significantly greater than the variance within treatment groups. The response appeared to be dose-related; the maximum effect (approximately 1.2 to 1.4 times the negative control SCE frequencies) was seen at 5000 µg/ml.

The test of acephate with metabolic activation involved exposing CHO cells to a higher concentration range for a shorter time (312.5 to 5000 µg/ml for 2 hours) than that used in the test without metabolic activation (125 to 2000 µg/ml for 21.5 hours. Therefore, direct comparisons are inappropriate. However, we conclude that acephate does not require metabolic activation to increase the SCE frequencies in CHO cells.

L5178Y Mouse Lymphoma (Tables 12-16). The initial concentration-range assay of acephate was performed in the absence and in the presence of activation with 18 concentrations between 1 and 1000 µg/ml. The samples exposed in the absence of a metabolic activation system were lost because of a technical error on the day following the exposure. The samples exposed in the presence of the metabolic activation system (Table 12) showed no significant cytotoxicity. Therefore, a repeat concentration-range assay was performed with and without activation with 10 concentrations between 1000 and 5000 µg/ml. In the absence of the metabolic activation system (Table 13), 5000 µg/ml yielded a relative total growth value of 44.9%, and 1000 µg/ml showed no significant cytotoxicity. The samples exposed in the presence of the metabolic activation system were lost because of an error in the preparation of the metabolic activation system. On the basis of the results in the absence of metabolic activation of the repeat assay, the mutagenesis assays were conducted both with and without metabolic activation to a maximum concentration of 5000 µg/ml. High cytotoxicity was not attained at 5000 µg/ml, but accurate testing at higher concentrations was not possible because of the viscosity of the concentrated acephate stock solutions.

Tables 14 and 15 present the results of mutagenesis testing of acephate in the absence of a metabolic activation system. At the highest concentration, 5000 µg/ml, the average relative total growth value was 55.7% in the first assay and 55.9% in the repeat assay. No significant depression in the relative total growth value was observed at or below 2500 µg/ml in the first assay and at 1000 µg/ml in the repeat assay. These concentrations yielded average relative total growth values of 99.2% and 87.9%, respectively. A concentration-related increase in induced mutation frequencies was observed from 2000 to 5000 µg/ml in the first assay and from 1000 to 5000 µg/ml in the second assay. Mutation frequencies at least twice the spontaneous frequency were observed for all the samples in the concentration range of 2000 to 5000 µg/ml (the eight highest concentrations) in both assays.

Table 16 presents the results of mutagenesis testing in the presence of the metabolic activation system. At the highest concentration of 5000 µg/ml, the average relative total growth value was 55.2%. No significant depression in the relative total growth value was observed at or below 2500 µg/ml, which yielded an average relative total growth value of 99.6%. From 2000 to 5000 µg/ml, a concentration-related increase in induced mutation was observed. A mutation frequency of at least twice the spontaneous frequency was observed for all samples in the concentration range of 3000 to 5000 µg/ml (the six highest concentrations).

These results indicate a mutagenic effect of acephate at the thymidine kinase locus in L5178Y mouse lymphoma cells, both without and with the metabolic activation system. In both cases, acephate increased the mutation frequency, in a concentration-related manner, above that of the solvent controls. The presence of the metabolic activation system did not appreciably affect the cytotoxic or mutagenic responses to acephate.

UDS in WI-38 Cells. Acephate was previously tested and found to be positive without activation and negative with activation (52).

Micronucleus Test (Tables 17, 18). The dosage and sacrifice schedule is shown in Table 17. The incidences of micronuclei in PCEs and the PCE-to-RBC ratios are given in Table 18. The vehicle control and acephate-treated groups were negative at all dose levels and sampling times ($\beta \leq 0.05$). The positive controls were positive ($\alpha < 0.05$). The elevated PCE-to-RBC ratios observed in all groups at 48 hours are thought to be an artifact. The pH of the stain was apparently lower than 6.8, giving very pale staining of mature cells, which were therefore difficult to count. The use of a new pH meter electrode on subsequent days eliminated the staining problem and the elevated ratios.

Although there were no fatalities in any group, the dose-related increase in weight loss in treated animals is a sign of increasing toxicity of the dosages used. Acephate meets all of the criteria for a nonmutagen in this test.

Table 5

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D7

ACEPHATE

Experiment 1

Compound	MA	% Conc.	Survivors Cells Per ml $(\times 10^{-7})$	Survivors %	Crossing-Over Mitotic Recombinants Per ml $(\times 10^{-3})$	Crossing-Over Mitotic Recombinants Per 10^5 Survivors	Crossing-Over Total Aberrants Per ml $(\times 10^{-3})$	Crossing-Over Total Aberrants Per 10^5 Survivors	Gene Conversion Convertants Per ml $(\times 10^{-1})$	Gene Conversion Convertants Per 10^5 Survivors	Reverse Mutation Revertants Per ml $(\times 10^{0})$	Reverse Mutation Revertants Per 10 Survivors
Negative Control												
DMSO	-	10.0	5.2	100	0	0	12	23	43	8.3	22	4.2
	+	10.0	5.7	100	2.5	4.4	12	21	54	9.5	22	3.9
Positive Control												
1,2,3,4-Diepoxybutane	-	0.013	4.6	88	97	210	810	1800	1700	370	350	76
	+	0.013	6.3	111	100	160	870	1400	1600	250	310	49
Acephate	-	1.0	3.5	67	3.0	8.6	18	51	62	18	31	8.9
	-	2.0	3.7	71	3.0	8.1	31	84	77	21	26	7.0
	-	3.0	2.7	52	8.0	30	38	140	91	34	20	7.4
	-	4.0	1.4	27	17	120	59	420	100	71	13	9.3
	-	5.0	1.3	25	20	150	53	410	83	64	4.0	3.1
	+	1.0	4.3	75	4.0	9.3	13	30	64	15	19	4.4
	+	2.0	2.9	51	3.0	10	41	140	70	24	23	7.9
	+	3.0	2.7	47	8.0	30	41	150	88	33	37	14
	+	4.0	3.4	60	18	53	57	170	110	32	37	11
	+	5.0	3.7	65	19	51	68	180	190	51	60	16

Table 6

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D7

ACEPHATE

Experiment 2

			Survivors		Crossing-Over				Gene Conversion		Reverse Mutation	
					Mitotic Recombinants		Total Aberrants		Convertants		Revertants	
Compound	MA	% Conc.	Cells Per ml ($\times 10^{-7}$)	%	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors	Per ml ($\times 10^{-1}$)	Per 10^6 Survivors	Per ml ($\times 10^{0}$)	Per 10^7 Survivors
Negative Control												
DMSO	-	10.0	5.9	100	0.50	.85	12	20	46	7.8	11	1.9
	+	10.0	6.1	100	0	0	13	21	36	5.9	6.0	0.98
Positive Control												
1,2,3,4-Diepoxybutane	-	0.013	4.1	69	160	390	660	1600	1000	240	180	44
	+	0.013	5.2	85	110	210	550	1100	1500	290	150	29
Acephate	-	3.0	4.5	76	10	22	62	140	120	27	11	2.4
	-	3.5	4.8	81	16	33	57	120	150	31	19	4.0
	-	4.0	4.7	80	18	38	100	210	150	32	21	4.5
	-	4.5	1.4	24	19	140	74	530	71	51	7.0	5.0
	-	5.0	0.73	12	9.0	120	61	840	47	64	6.0	8.2
	+	3.0	6.6	108	3.0	4.5	54	8.2	140	21	11	1.7
	+	3.5	5.8	95	4.0	6.9	74	130	160	28	15	2.6
	+	4.0	6.1	100	17	28	62	100	160	26	22	3.6
	+	4.5	4.8	79	13	27	79	160	170	35	24	5.0
	+	5.0	3.2	52	13	41	84	260	150	47	8.0	2.5

Table 7

DIFFERENTIAL TOXICITY ASSAY OF ACEPHATE WITH SALMONELLA TYPHIMURIUM STRAINS

Experiment 1

Compound	Amount of Compound Applied to Disc	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		SL4525 (rec$^+$)		SL4700 (rec$^-$)		TA1978 (uvrB$^+$)		TA1538 (uvrB$^-$)	
Negative Control									
Kanamycin	100 µg	15	16	15	15	14	15	15	16
Positive Controls									
MMS*	10 mg	53	53	70	70				
4-NQO†	2 mg					12	12	24	25
Acephate	1 mg	6	6	6	6	6	6	6	6
	5 mg	9	9	10	10	7	7	7	7
Negative Control		Experiment 2							
Kanamycin	100.0 µg	15	14	16	15	17	17	15	16
Positive Controls									
MMS*	10.0 mg	45	40	72	74				
4-NQO†						12	12	28	26
Acephate	5.0 mg	7	8	10	10	8	8	8	8
	10.0 mg	9	9	12	12	9	9	10	9

---

* MMS = Methylmethanesulfonate.

† 4-NQO = 4-Nitroquinoline-1-oxide.

Table 8

CYTOTOXICITY TEST OF CHINESE HAMSTER OVARY CELLS EXPOSED TO ACEPHATE FOR 21.5 HOURS WITHOUT METABOLIC ACTIVATION

Treatment	Number of Divisions in Bromodeoxyuridine (BrdU)[*]
Negative control (0.95% ethanol)	M_2
Acephate (μg/ml)	
8	M_2
40	M_2
200	M_2
1000	M_1
5000	--[†]
Positive control (10^{-3} M ethyl methanesulfonate)	M_2

---

[*] M_1, one division in BrdU; M_2, two divisions in BrdU.

[†] This culture was not harvested because of excessive cytoxicity.

Table 9

CYTOTOXICITY TEST OF CHINESE HAMSTER OVARY CELLS EXPOSED TO ACEPHATE FOR 2 HOURS WITH METABOLIC ACTIVATION

Treatment	Number of Divisions in Bromodeoxyuridine (BrdU)*
Negative control (0.95% ethanol)	M_2
Acephate (µg/ml)	
8	M_2
40	M_2
200	M_2
1000	$M_1 \cong M_2$
5000	M_2
Positive control (10^{-3} M dimethylnitrosamine)	M_2

---

*M_1, one division in BrdU; M_2, two divisions in BrdU.

Table 10

SISTER CHROMATID EXCHANGE (SCE) FREQUENCIES IN CHINESE HAMSTER OVARY (CHO) CELLS EXPOSED TO ACEPHATE FOR 21.5 HOURS WITHOUT METABOLIC ACTIVATION

Treatment	Scorer*	No. of SCEs	No. of Chromosomes	Chromosomes/Cell (Mean ± SD)	SCEs/Cell[†] (Mean ± SE)	SCEs/ Chromosome[†] (Mean ± SE)
Negative control (0.95% ethanol)	A	382	476	19.04 ± 1.21	15.28 ±0.78	0.803 ± 0.041
	B	267	497	19.88 ± 0.60	10.68 ±0.65	0.537 ± 0.033
Acephate (µg/ml)						
125	A	460	478	19.12 ± 1.30	18.40 ± 0.86	0.962 ± 0.045
	B	359	491	19.64 ± 1.29	14.36 ± 0.76	0.731 ± 0.039
250	A	420	477	19.08 ± 1.15	16.80 ± 0.82	0.881 ± 0.043
	B	474	497	19.88 ± 0.83	18.96 ± 0.87	0.954 ± 0.044
500	A	609	486	19.44 ± 1.08	24.36 ± 0.99	1.253 ± 0.051
	B	591	497	19.88 ± 0.83	23.64 ± 0.97	1.189 ± 0.049
1000	A	754	474	18.96 ± 1.17	30.16 ± 1.10	1.591 ± 0.058
	B	704	503	20.12 ± 0.88	28.16 ± 1.06	1.400 ± 0.053
2000	A	1232	473	18.92 ± 1.35	49.28 ± 1.40	2.605 ± 0.074
	B	1355	500	20.00 ± 0.71	54.20 ± 1.47	2.710 ± 0.074
Positive control (10^{-3} ethyl methanesulfonate)	A	854	486	19.44 ± 1.50	34.16 ± 1.17	1.757 ± 0.060
	B	1064	500	20.00 ± 0.71	42.56 ± 1.31	2.128 ± 0.065

---

*Each scorer analyzed 25 cells per sample.

[†] A one-way analysis of variance comparing the SCE frequencies in CHO cells exposed to acephate and to the negative control indicated that the variance between treatment groups was significantly greater ($P < 0.0005$) than the variance within treatment groups.

Table 11

SISTER CHROMATID EXCHANGE (SCE) FREQUENCIES IN CHINESE HAMSTER OVARY (CHO) CELLS EXPOSED TO ACEPHATE FOR 2 HOURS WITH METABOLIC ACTIVATION

Treatment	Scorer[*]	No. of SCEs	No. of Chromosomes	Chromosomes/Cell (Mean ± SD)	SCEs/Cell[†] (Mean ± SE)	SCEs/Chromosome[†] (Mean ± SE)
Negative control (.95% ethanol)	A	479	489	19.56 ± 0.92	19.16 ± 0.88	0.980 ± 0.045
	B	526	469	18.76 ± 0.78	21.04 ± 0.92	1.222 ± 0.049
Acephate (μg/ml)						
312.5	A	426	485	19.40 ± 0.91	17.04 ± 0.83	0.878 ± 0.043
	B	397	478	19.12 ± 1.09	15.88 ± 0.80	0.831 ± 0.042
625	A	431	499	19.96 ± 0.45	17.24 ± 0.83	0.864 ± 0.041
	B	451	471	18.84 ± 0.99	18.04 ± 0.85	0.958 ± 0.045
1250	A	553	494	19.76 ± 0.60	22.12 ± 0.94	1.119 ± 0.048
	B	496	479	19.16 ± 1.21	19.84 ± 0.89	1.035 ± 0.046
2500	A	575	477	19.08 ± 0.70	23.00 ± 0.96	1.206 ± 0.050
	B	505	471	18.84 ± 0.69	20.20 ± 0.90	1.072 ± 0.048
5000	A	695	498	19.92 ± 1.22	27.80 ± 1.05	1.396 ± 0.053
	B	697	483	19.32 ± 0.75	27.88 ± 1.06	1.443 ± 0.055
Positive control (10^{-3} M dimethylnitrosamine)	A	1050	492	19.68 ± 0.80	42.00 ± 1.30	2.134 ± 0.066
	B	898	477	19.08 ± 1.19	35.92 ± 1.20	1.883 ± 0.063

---

[*] Each scorer analyzed 25 cells per sample.

[†] A one-way analysis of variance comparing the SCE frequencies in CHO cells exposed to acephate and to the negative control indicated that the variance between the treatment groups was significantly greater ($P < 0.005$) than the variance within treatment groups.

Table 12

MOUSE LYMPHOMA FORWARD MUTATION CONCENTRATION-RANGE ASSAY OF ACEPHATE IN THE PRESENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency ($\times 10^{-6}$)	Induced Mutation Frequency ($\times 10^{-6}$)
Solvent control (1% DMSO)	103.8	600	613	172	102.7	106.6	56	--
	96.2	600	581	173	97.3	93.6	60	--
Positive control (5 μg/ml, 3-MCA)	70.9	600	408	497	68.3	48.5	244	186
	73.1	600	476	539	79.7	58.3	226	168
Acephate								
1	105.9	600	548	136	91.8	97.2	50	--
2	105.9	600	416	155	69.7	73.8	75	17
5	96.2	600	504	167	84.4	81.2	66	8
10	105.9	600	495	150	82.9	87.8	61	3
20	101.9	600	551	194	92.3	94.0	70	12
40	105.9	600	437	172	73.2	77.5	79	21
80	105.9	600	520	168	87.1	92.2	65	7
100	105.9	600	561	279	94.0	99.5	99	41
150	110.0	600	593	276	99.3	109.3	93	35
200	105.9	600	559	195	93.6	99.2	70	12
300	105.9	600	529	218	88.6	93.8	82	24
400	110.0	600	487	217	81.6	89.7	89	31
500	105.9	600	520	179	87.1	92.2	69	11
600	105.9	600	381	163	63.8	67.6	86	28
700	105.9	600	550	256	92.1	97.6	93	35
800	101.9	600	396	190	66.3	67.6	96	38
900	101.9	600	539	255	90.3	92.0	95	37
1000	101.9	600	619	317	103.1	105.7	102	44

Table 13

MOUSE LYMPHOMA FORWARD MUTATION CONCENTRATION-RANGE ASSAY OF ACEPHATE IN THE ABSENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency ($\times 10^{-6}$)	Induced Mutation Frequency ($\times 10^{-6}$)
Solvent control (1% DMSO)	100.0	600	580	138	107.3	107.3	48	--
	100.0	600	501	134	92.7	92.7	53	--
Positive control (500 μg/ml EMS)	58.5	600	387	1044	71.6	41.9	540	489
	52.2	600	406	1037	75.1	39.2	511	460
Acephate								
1000	100.1	600	585	205	108.2	108.3	70	19
1500	91.8	600	506	183	93.6	85.9	72	21
2000	82.4	600	563	325	104.2	85.8	115	64
2500	75.1	600	584	322	108.2	81.3	110	59
3000	72.5	600	564	322	104.3	75.7	114	63
3500	72.5	600	460	409	85.1	61.7	178	127
4000	72.0	600	493	387	91.2	65.7	157	106
4500	61.0	600	492	401	91.0	55.5	163	112
4700	63.4	600	489	399	90.5	57.4	163	112
5000	58.5	600	415	398	76.8	44.9	192	141

Table 14

MOUSE LYMPHOMA FORWARD MUTATION ASSAY OF ACEPHATE IN THE ABSENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency ($\times 10^{-6}$)	Induced Mutation Frequency ($\times 10^{-6}$)
Solvent control (1% DMSO)	95.8	600	599	112	104.7	100.3	37	--
	104.2	600	545	166	95.3	99.3	61	--
Positive control (500 μg/ml EMS)	70.9	600	377	1012	65.9	46.7	537	488
	73.2	600	308	1007	53.8	39.4	654	605
Acephate								
1000	108.6	NC*	--	--	--	--	--	--
	108.6	NC	--	--	--	--	--	--
1500	103.8	NC	--	--	--	--	--	--
	99.6	NC	--	--	--	--	--	--
2000	103.8	600	564	338	98.6	102.3	120	71
	103.8	600	524	287	91.6	95.1	110	61
2500	99.7	600	524	334	91.6	91.3	127	78
	103.8	600	590	443	103.1	107.1	150	101
3000	99.7	600	504	365	88.1	87.8	145	96
	108.7	600	522	344	91.3	99.2	132	83
3500	91.6	600	501	406	87.6	80.2	162	113
	99.1	600	--†	370	--	--	--	--
4000	78.8	600	466	435	81.5	64.2	187	138
	91.6	600	496	465	86.7	79.4	188	139
4500	78.8	600	425	467	74.3	58.5	220	171
	88.3	600	401	519	80.6	71.2	225	176
4700	81.7	600	398	485	69.6	56.8	244	195
	85.0	600	370	463	64.7	55.0	250	201
5000	85.0	600	342	530	59.8	50.8	310	261
	81.9	600	423	472	74.0	60.6	223	174

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[*] Not cloned.

[†] Sample lost.

Table 15

REPEAT MOUSE LYMPHOMA FORWARD MUTATION ASSAY OF ACEPHATE IN THE ABSENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (µg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency ($\times 10^{-6}$)	Induced Mutation Frequency ($\times 10^{-6}$)
Solvent control (1% DMSO)	100.0	600	567	122	101.1	101.1	43	--
	100.0	600	555	151	98.9	98.9	54	--
Positive control (500 µg/ml EMS)	59.9	600	305	958	54.4	32.6	628	579
	64.2	600	326	955	58.1	37.3	586	537
Acephate								
1000	99.3	600	546	219	97.3	96.6	80	31
	91.4	600	486	206	86.6	79.2	85	36
1500	91.0	600	564	189	82.7	75.3	81	32
	95.0	600	497	204	88.6	84.2	82	33
2000	91.2	600	539	282	96.1	87.6	105	56
	91.2	600	476	269	84.8	77.4	113	64
2500	91.2	600	510	282	90.9	82.9	111	62
	87.4	600	525	277	93.6	81.8	106	57
3000	80.4	600	468	328	83.4	67.1	140	91
	87.4	600	441	341	78.6	68.7	155	106
3500	80.7	600	460	364	82.0	66.2	158	109
	77.6	600	429	320	76.5	59.2	149	100
4000	77.4	600	425	378	75.8	58.6	178	129
	77.4	600	449	382	80.0	61.9	170	121
4500	71.7	600	441	385	78.6	56.4	175	126
	74.5	600	430	396	76.6	57.1	184	135
4700	71.7	600	504	392	89.8	64.4	156	107
	71.7	600	475	406	84.7	60.7	171	122
5000	69.1	600	469	422	83.6	57.8	180	131
	71.7	600	469	392	83.6	59.9	167	118

Table 16

MOUSE LYMPHOMA FORWARD MUTATION ASSAY OF ACEPHATE IN THE PRESENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (µg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency ($\times 10^{-6}$)	Induced Mutation Frequency ($\times 10^{-6}$)
Solvent control (1% DMSO)	97.0	600	564	227	103.1	100.0	81	--
	103.0	600	530	215	96.9	99.8	81	--
Positive control (5 µg/ml 3-MCA)	40.3	600	244	491	44.6	18.0	402	321
	35.8	600	249	445	45.5	16.3	357	276
Acephate								
1000	98.3	NC*	--	--	--	--	--	--
	79.6	NC	--	--	--	--	--	--
1500	99.8	NC	--	--	--	--	--	--
	103.0	NC	--	--	--	--	--	--
2000	106.4	600	483	305	88.3	94.0	126	45
	84.2	600	356	303	65.1	54.8	170	89
2500	97.0	600	479	346	87.6	84.9	144	63
	112.8	600	554	364	101.3	114.2	131	50
3000	106.4	600	416	358	76.1	80.9	172	91
	93.9	600	473	401	86.5	81.2	170	89
3500	75.2	600	511	445	93.4	70.3	174	93
	72.1	600	543	401	99.3	71.6	148	67
4000	90.3	600	420	430	76.8	69.3	205	124
	72.6	600	446	409	81.5	59.2	183	102
4500	76.6	600	388	389	70.9	54.3	201	120
	68.1	600	460	405	84.1	57.3	176	95
4700	75.5	600	412	375	75.3	56.9	182	101
	71.8	600	356	361	65.1	46.7	203	122
5000	72.6	600	520	420	95.1	69.0	162	81
	69.1	600	328	437	60.0	41.4	266	185

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*Not cloned.

Table 17

DOSAGE AND SACRIFICE SCHEDULE FOR ACEPHATE

Compound	No. Animals	Dose		Mean Body Weight Change (g)	No. Sacrificed			No. Fatalities
		0 Hr	24 Hr		48 Hr	72 Hr	96 Hr	
TMP--Positive control	8	1 g/kg	1 g/kg	-1.6	8	--	--	0
H_2O--Vehicle control	24	5 ml/kg	5 ml/kg	-1.1*	8	8	8	0
Acephate								
Low Dose	24	75 mg/kg	75 mg/kg	-0.2	8	8	8	0
Medium Dose	24	150 mg/kg	150 mg/kg	-0.4	8	8	8	0
High Dose	24	300 mg/kg	300 mg/kg	-1.2	8	8	8	0

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*Eight animals (2 cages of 4 each) lost 4 grams each. The water supply was possibly unreachable, so that the animals refused to eat. The remaining 16 animals had a mean weight gain of 0.4 grams.

Table 18

ACEPHATE
INCIDENCE OF MICRONUCLEATED POLYCHROMATOPHILIC ERYTHROCYTES*
(500 PCE's Scored per Animal)

Dose	48 Hr Animal No.	48 Hr PCEs with MN	48 Hr PCE/RBC	72 Hr Animal No.	72 Hr PCEs with MN	72 Hr PCE/RBC	96 Hr Animal No.	96 Hr PCEs with MN	96 Hr PCE/RBC
Water 5 ml/kg (vehicle control)	54ø	0	2.20	55ø	1	1.05	53ø	1	0.82
	54r	1	1.57	55r	1	0.89	53r	1	0.86
	54ℓ	2	1.60	55ℓ	0	0.93	53ℓ	0	0.41
	54b	2	1.79	55b	2	1.35	53b	0	0.53
	56ø	0	2.00	58ø	0	1.23	57ø	0	1.01
	56r	1	2.22	58r	1	1.05	57r	0	1.00
	56ℓ	0	1.34	58ℓ	2	0.79	57ℓ	0	0.84
	56b	1	1.35	58b	1	0.85	57b	0	1.54
	Total	7			8			2	
	Average		1.76			1.02			0.75
Acephate 75 mg/kg	61ø	0	1.69	59ø	1	0.99	60ø	0	2.64
	61r	0	1.12	59r	2	1.02	60r	0	1.48
	61ℓ	1	1.74	59ℓ	0	1.60	60ℓ	0	2.47
	61b	2	1.47	59b	0	1.51	60b	0	1.32
	63ø	2	1.87	62ø	0	0.91	64ø	2	0.93
	63r	1	1.67	62r	1	2.11	64r	1	1.16
	63ℓ	1	1.31	62ℓ	1	1.45	64ℓ	2	0.95
	63b	0	2.08	62b	1	0.71	64b	0	1.15
	Total	7			6			5	
	Average		1.62			1.29			1.51
Acephate 150 mg/kg	65ø	0	2.26	66ø	1	0.79	67ø	0	1.85
	65r	2	2.00	66r	1	0.80	67r	0	1.13
	65ℓ	1	1.90	66ℓ	0	0.89	67ℓ	0	0.56
	65b	1	1.87	66b	1	0.51	67b	2	0.83
	69ø	1	3.28	68ø	1	1.09	70ø	0	0.93
	69r	1	1.70	68r	0	1.89	70r	1	1.29
	69ℓ	0	1.95	68ℓ	0	1.54	70ℓ	1	0.83
	69b	1	1.47	68b	2	1.10	70b	2	0.51
	Total	7			6			6	
	Average		2.05			1.08			0.99

continued

Table 18 (concluded)

	48 Hr			72 Hr			96 Hr		
Dose	Animal No.	PCEs with MN	PCE/RBC	Animal No.	PCEs with MN	PCE/RBC	Animal No.	PCEs with MN	PCE/RBC
Acephate 300 mg/kg	74ø	1	1.76	71ø	1	1.01	73ø	0	1.25
	74r	1	1.96	71r	4	0.43	73r	0	1.68
	74ℓ	2	1.34	71ℓ	0	0.71	73ℓ	2	1.03
	74b	1	1.67	71b	0	1.46	73b	0	2.35
	76ø	0	0.82	72ø	1	1.15	75ø	1	1.46
	76r	1	2.53	72r	1	1.12	75r	1	1.14
	76ℓ	0	1.94	72ℓ	0	0.95	75ℓ	1	1.69
	76b	0	1.45	72b	0	1.23	75b	1	0.98
	Total	6			7			6	
	Average		1.68			1.01			1.45
TMP† 1000 mg/kg	50ø	4	1.91						
	50r	12	0.88						
	50ℓ	7	1.42						
	50b	8	1.14						
	51ø	7	1.44						
	51r	4	1.16						
	51ℓ	4	1.06						
	51b	4	0.82						
	Total	50							
	Average		1.23						

---

*PCE = polychromatophilic erythrocyte; RBC = mature erythrocyte; MN = micronuclei.

†This positive control group is the same group reported with the micronucleus test of monocrotophos, which was done at the same time as this test.

Azinphos-Methyl

S. typhimiurium Plate Incorporation (Table 19). This compound was previously tested and found nonmutagenic both with and without activation in strains TA1535, TA1537 and TA1538 (51). To complete the standard five-strain assay, it was tested under the present contract in strains TA98 and TA100 and was found nonmutagenic both with and without activation.

E. coli WP-2. Azinphos-methyl was found nonmutagenic, both with and without activation in previous testing (51).

S. cerevisiae D3 (Table 20). This compound was previously evaluated over a narrow dose range and was reported as recombinogenic, both with and without activation (51). It was retested over a wider dose range under the present contract, and the results indicate that it is recombinogenic without activation, but is not recombinogenic with activation.

S. cerevisiae D7 (Tables 21, 22). A concentration range of 0.05 to 4.0% was chosen for the first experiment and 0.1 to 4.0% for the second. No reproducible, dose-related increase in the number of aberrant-type colonies, tryptophan convertants, or isoleucine revertants was observed. Solubility problems precluded attainment of a more toxic dose.

E. coli/B. subtilis Relative Toxicity. Azinphos-methyl was previously tested and reported to be negative in both species (51). Reexamination of the data indicates that the results are more appropriately categorized as inconclusive.

S. typhimurium Relative Toxicity (Tables 23, 24). This compound was negative in both strain pairs.

SCE in CHO Cells (Tables 25-29). In the cytotoxicity test with metabolic activation, the culture medium foamed when azinphos-methyl was added at a concentration of 1000 μg/ml or greater; in the cytotoxicity test without metabolic activation, azinphos-methyl precipitated in culture medium at a concentration of 1000 μg/ml or higher.

Table 25 presents the results of the cytotoxicity test of azinphos-methyl in CHO cell cultures without metabolic activation. At a concentration of 200 μg/ml or greater, azinphos-methyl was cytotoxic to CHO cells. At 40 μg/ml or less, M_2 metaphase cells were present. Table 26 presents the results of the cytotoxicity test of azinphos-methyl in CHO cell cultures with metabolic activation. At a concentration of 1000 μg/ml or greater, azinphos-methyl was cytotoxic to CHO cells. At 200 μg/ml or less M_2 metaphase cells were present.

For the SCE test without metabolic activation, slides from two concentrations of the test compound were evaluated, both in the initial test and the repeat test. For the test with metabolic activation, slides from four concentrations of the test compound were evaluated. Table 27 presents the SCE frequencies in CHO cells exposed to azinphos-methyl without metabolic activation. Cell cultures exposed to 80 μg/ml could not be harvested because of excessive cytotoxicity. Cell cultures exposed to 5 μg/ml azinphos-methyl could not be evaluated because they were lost during harvest. In cultures exposed to 20 μg/ml and 40 μg/ml, duplicate samples exhibited inconsistent cell cycle effects. At each concentration one cytogeneticist evaluated 25 cells and the other was unable to evaluate 25 cells because of insufficient M_2 metaphase cells. Because cytogeneticist B was able to evaluate only 9 cells exposed to 20 µg/ml azinphos-methyl, the SCE frequencies of CHO cells exposed to this concentration could not be included in the analysis of variance. Therefore, only cells exposed to two concentrations of azinphos-methyl in addition to the negative controls were available for statistical analysis. These results were considered insufficient for a meaningful statistical evaluation. However, no marked increase in SCE frequencies correlated with increasing concentrations of azinphos-methyl was observed.

The SCE test without metabolic activation was repeated; Table 28 presents these results. In cell cultures exposed to 5 µg/ml azinphos-methyl, one cytogeneticist evaluated 25 cells and the other was unable to evaluate 25 cells because of poor contrast in the staining of the chromosomes. In cell cultures exposed to 40 µg/ml and 80 µg/ml, inconsistent cell cycle effects were once again observed; at each concentration one cytogeneticist was able to evaluate 25 cells and the other was unable to find sufficient M_2 metaphase cells for analysis. As in the initial test of azinphos-methyl without metabolic activation, only two concentrations of azinphos-methyl-treated cells were available for statistical evaluation by analysis of variance. Again, these results were considered insufficient for a meaningful statistical evaluation. In this test, cytogeneticist B did observe an elevated SCE frequency in CHO cells exposed to 80 µg/ml azinphos-methyl; no other marked elevations in SCE frequencies among treated cells were observed. The lack of a scorable duplicate sample makes interpretation of the single elevated SCE frequency at 80 µg/ml difficult, especially because the positive control samples evaluated by cytogeneticist B in this experiment gave an unexpectedly low SCE frequency. The possibility that the two samples were confused at harvest time cannot be eliminated.

The SCE tests of azinphos-methyl in CHO cells without metabolic activation must be considered inconclusive. The non-reproducible cell cycle effects and poor contrast in prepared slides may be due to the effects of azinphos-methyl.

Table 29 presents the SCE frequencies in CHO cells exposed to azinphos-methyl with metabolic activation. Cell cultures exposed to 400 µg/ml could not be harvested because of excessive cytotoxicity. A one-way analysis of variance was conducted on SCE frequencies for the four lower concentrations of azinphos-methyl and of the negative controls. The variance between treatment groups was not significantly greater than the variance within treatment groups.

These findings indicate that azinphos-methyl did not induce SCE's in CHO cells with metabolic activation and produced inconclusive results without metabolic activation.

L5178Y Mouse Lymphoma Assay (Tables 30-33). The results of concentration-range testing of azinphos-methyl in the absence of the metabolic activation system (Table 30) indicated that the range to use in the mutagenesis assay was approximately 5 to 80 µg/ml (94.2% relative total growth at 5 µg/ml and 1.3% relative total growth at 80 µg/ml). Results of concentration-range tests of azinphos-methyl in the presence of the metabolic activation system (Table 31) showed that the approximate range to use in the mutagenesis assay was less than 1 μg/ml to somewhat more than 100 μg/ml (75.7% relative total growth at 1 μg/ml and 21.7% relative total growth at 100 μg/ml.

Table 32 presents the results of mutagenesis testing of azinophos-methyl in the absence of the metabolic activation system. Azinphos-methyl was extremely toxic at concentrations greater than 45 μg/ml, yielding relative total growth values of less than 9.0%. No significant depression in the relative total growth was observed at a concentration of 9 μg/ml or less. At 9 μg/ml, the average relative total growth was 97.1%. Over the tested range, a concentration-related increase in induced mutation frequency was observed. However, none of the concentrations yielding average relative growth values of greater than 10.0% showed a mutation frequency twice the spontaneous frequency.

Table 33 presents the results of mutagensis testing of azinphos-methyl in the presence of the metabolic activation system. Azinphos-methyl was extremely toxic at concentrations greater than 108 μg/ml, with relative suspension growth values of less than 8.0%. The lowest concentration, 6 μg/ml, yielded an average relative total growth of 63.0%. From 8 to 15 μg/ml, a concentration-related increase in induced mutation frequency was observed. The average mutation frequency at 10 μg/ml was three times the spontaneous level, and at 15 μg/ml it was over five times the average frequency observed with the solvent control. However, at 6 μg/ml the average mutation frequency was elevated to a value that did not follow the concentration-related response observed from 8 to 15 μg/ml. We were not able to determine the reason for this observation.

These combined results indicate a mutagenic effect of azinphos-methyl in the presence, but not in the absence, of the metabolic activation system. With metabolic activation, azinphos-methyl increased the mutation frequency--in a concentration-related manner over a limited range of concentrations--above the frequencies observed with the solvent controls, and a mutagenic effect was clearly indicated by the unusually high induced mutation frequencies observed with the two highest concentrations within this range. However, additional testing is needed to determine potential mutagenicity between 45 and 54 μg/ml in the absence of actiation, and repeat testing is needed in the presence of activation to more precisely define concentration-related effects.

UDS in WI-38 Cells (Tables 34-36). This compound was previously tested and reported to be negative without activation and positive with activation (51). Because the control data for the previous test appeared suspiciously low, the compound was retested once without activation and twice with activation. The results of this retest without activation are consistent with the previous test, and indicate the compound is negative. The results of the retests with activation indicate that the compound is negative, as opposed to the earlier positive interpretation. Since the retest control values are consistent with our historical control values, we conclude that the compound is negative in this test system both with and without activation.

Micronucleus Test (Tables 37, 38). The dosage and sacrifice schedule is shown in Table 37. The results for all vehicle control and azinphos-methyl-treated groups (Table 38) were negative ($\beta < 0.05$); the positive control (TMP-treated) group was positive ($\alpha < 0.001$). The mean ratio of PCEs to RBCs was within the historical control range (0.5-1.5) for all dose-time groups. The values obtained for the blood smears do not appear to have any direct relationship to the values observed in the bone marrow. The vehicle control and compound-treated group totals are within the normal range for micronucleated cells in bone marrow, and the TMP group is clearly positive. The dose-related weight loss observed 24 hours after the first dose, along with the fatalities in the high-dose group, indicates a maximum tolerated dose was administered.

Mouse Dominant Lethal Test. Azinphos-methyl was previously tested and found negative in this test system (51).

Table 19

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

AZINPHOS-METHYL
Retest

Compound	Metabolic Activation	Compound Added (µg/plate)	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative Control							
DMSO	-					10 13	100 105
	+					32 29	90 116
Positive Control							
2-Anthramine	-	1.0				25	105
	+	1.0				273	488
Sodium Azide	-	0.5					474
2-Nitrofluorene	-	5.0				466	
Azinphos-methyl	-	500.0				15	97
	-	1,000.0				19	115
	-	2,000.0				24	21T*
	-	3,000.0				19	T
	-	4,000.0				14	T
	-	5,000.0				19	T
	+	500.0				23	102
	+	1,000.0				24	124
	+	2,000.0				16	39T
	+	3,000.0				20	T
	+	4,000.0				19	T
	+	5,000.0				16	T

---

*T, toxic.

Table 20

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

AZINPHOS-METHYL
Retest

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml (x 10^{-7})	Percent	Per ml (x 10^{-3})	Per 10^5 Survivors
Negative Control: DMSO	-		8.0	100	4.0	5.0
	+		7.9	100	3.5	4.4
Positive Control 1,2,3,4-Diepoxybutane	-	0.025	7.8	98	902.5	1157.1
	+	0.025	C*	C	C	C
Azinphos-methyl	-	1	7.2	90	9.0	12.5
	-	2	7.9	99	9.0	11.4
	-	3	7.9	99	5.0	5.3
	-	4	6.8	85	7.0	10.3
	-	5	5.4	68	18.0	33.3
	+	1	8.7	110	18.0	20.7
	+	2	7.7	97	9.0	11.7
	+	3	8.4	106	11.0	13.1
	+	4	6.6	84	8.0	12.1
	+	5	5.9	75	10.0	17.0

* C, contaminated.

Table 21

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D7

AZINPHOS-METHYL

Experiment 1

Compound	MA	% Conc.	Survivors Cells Per ml (× 10^{-7})	Survivors %	Crossing-Over Mitotic Recombinants Per ml (× 10^{-3})	Crossing-Over Mitotic Recombinants Per 10^5 Survivors	Crossing-Over Total Aberrants Per ml (× 10^{-3})	Crossing-Over Total Aberrants Per 10^5 Survivors	Gene Conversion Convertants Per ml (× 10^{-1})	Gene Conversion Convertants Per 10^6 Survivors	Reverse Mutation Revertants Per ml (× 10^0)	Reverse Mutation Revertants Per 10^7 Survivors
Negative Control												
DMSO	-		6.0	100	0	0	17	28	85	14	42	7.0
	+		5.5	100	1.0	1.8	14	25	81	15	30	5.5
Positive Controls												
1,2,3,4-Diepoxybutane	-	0.006	5.5	92	55	100	1100	2000	2600	470	600	110
	+	0.006	7.0	130	41	59	510	730	1800	260	270	39
Azinphos-Methyl	-	0.05	5.3	88	1.0	1.9	13	25	36	6.8	14	2.6
	-	0.5	3.8	63	0	0	4.0	9.9	100	26	41	11
	-	1.0	4.7	78	2.0	4.3	58	120	110	23	35	7.5
	-	2.0	3.2	53	2.0	6.3	47	150	130	41	37	12
	-	4.0	3.4	57	0	0	31	91	110	32	36	11
	+	0.05	4.6	84	0	0	1.0	2.2	82	18	41	8.9
	+	0.5	5.4	98	0	0	19	35	46	8.5	5.0	0.9
	+	1.0	5.2	95	0	0	15	29	99	19	38	7.3
	+	2.0	4.5	82	0	0	58	130	70	16	5.0	1.1
	+	4.0	4.4	80	2.0	4.5	47	110	110	25	38	8.6

Table 22

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D7

AZINPHOS-METHYL

Experiment 2

Compound	MA	% Conc.	Survivors: Cells Per ml ($\times 10^{-7}$)	Survivors: %	Crossing-Over: Mitotic Recombinants: Per ml ($\times 10^{-3}$)	Crossing-Over: Mitotic Recombinants: Per 10^5 Survivors	Crossing-Over: Total Aberrants: Per ml ($\times 10^{-3}$)	Crossing-Over: Total Aberrants: Per 10^5 Survivors	Gene Conversion: Convertants: Per ml ($\times 10^{-1}$)	Gene Conversion: Convertants: Per 10^6 Survivors	Reverse Mutation: Revertants: Per ml ($\times 10^0$)	Reverse Mutation: Revertants: Per 10^7 Survivors
Negative Control												
DMSO	-		5.0	100	1.0	2.0	19	38	53	11	8.0	0.8
	+		5.5	100	1.0	1.8	19	35	72	13	6.0	1.1
Positive Controls												
1,2,3,4-Diepoxybutane	-	0.006	4.5	90	70	160	800	1,800	1,300	290	270	60
	+	0.006	6.6	120	63	95	690	1,000	800	120	100	15
Azinphos-Methyl	-	0.1	6.6	130	0	0	19	29	110	17	31	4.7
	-	0.5	5.3	110	1.0	1.9	45	85	110	21	44	8.3
	-	1.0	5.5	110	1.0	1.8	39	71	130	24	48	8.7
	-	2.0	4.2	84	4.0	9.5	57	140	120	29	48	11
	-	4.0	4.4	88	0	0	0	0	140	32	65	15
	+	0.1	6.3	130	2.0	3.2	35	56	98	16	27	4.3
	+	0.5	5.0	91	0	0	20	40	130	26	55	11
	+	1.0	5.1	93	1.0	2.0	39	76	140	27	54	11
	+	2.0	5.1	93	1.0	2.0	38	75	140	27	100	20
	+	4.0	4.4	80	1.0	2.3	35	80	160	36	59	13

Table 23

DIFFERENTIAL TOXICITY ASSAYS WITH SALMONELLA TYPHIMURIUM STRAINS

Experiment 1

Compound	Amount of Compound Applied to Disc	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		SL4525 (rec+)		SL4700 (rec-)		TA1978 (uvrB+)		TA1538 (uvrB-)	
Negative Control									
Kanamycin	100.0 µg	14	13	13	14	13	13	14	14
Positive Controls									
MMS*	10.0 mg	47	46	68	65				
4-NQO†	2.0 mg					12	12	24	23
Azinphos-Methyl	1.0 mg	9	9	9	9	9	8	8	8
	5.0 mg	9	9	9	9	9	8	9	8

Table 24

DIFFERENTIAL TOXICITY ASSAYS WITH SALMONELLA TYPHIMURIUM STRAINS

Experiment 2

Compound	Amount of Compound Applied to Disc	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		SL4525 (rec$^+$)		SL4700 (rec$^-$)		TA1978 (uvrB$^+$)		TA1538 (uvrB$^-$)	
Negative Control									
Kanamycin	100.0 µg	15	14	16	15	17	17	15	16
Positive Controls									
MMS*	10.0 mg	45	40	72	74				
4-NQO†	2.0 mg					12	12	28	26
Azinphos-Methyl	5.0 mg	8	8	9	10	9	8	8	8
	10.0 mg	9	8	10	10	10	11	9	9

Table 25

CYTOTOXICITY TEST OF CHINESE HAMSTER OVARY CELLS EXPOSED TO AZINPHOS-METHYL FOR 21.5 HOURS WITHOUT METABOLIC ACTIVATION

Treatment	Number of Divisions in Bromodeoxyridine (BrdU)[*]
Negative Control (1% DMSO)	M_2
Azinphos-methyl (μg/ml)	
8	M_2
40	M_2
200	--[†]
1000[‡]	--[†]
5000[‡]	--[†]
Positive control (10^{-3} M ethyl methanesulfonate)	M_2

---

[*] Two divisions in BrdU.

[†] Cultures were not harvested because of excessive cytotoxicity.

[‡] Azinphos-methyl precipitated in culture medium at these concentrations.

Table 26

CYTOTOXICITY TEST OF CHINESE HAMSTER OVARY CELLS EXPOSED TO AZINPHOS-METHYL FOR 2 HOURS WITH METABOLIC ACTIVATION

Treatment	Number of Divisions in Bromodeoxyridine (BrdU)[*]
Negative control (1% DMSO)	M_2
Azinphos-methyl (μg/ml)	
8[†]	M_2
40	M_2
200	M_2
1000[‡]	--§
5000[‡]	--§
Positive control (10^{-3} M dimethylnitrosamine)	$M_2 > M_1$[**]

---

[*] Two divisions in BrdU.

[†] Less than 8 μg/ml azinphos-methyl may have been delivered.

[‡] The culture medium foamed when azinphos-methyl was added at these concentrations.

§Cultures were not harvested because of excessive cytotoxicity.

[**] The cell type to the left of the > sign was in excess.

Table 27

SISTER CHROMATID EXCHANGE (SCE) FREQUENCIES IN CHINESE HAMSTER OVARY (CHO) CELLS EXPOSED TO AZINPHOS-METHYL FOR 21.5 HOURS WITHOUT METABOLIC ACTIVATION (FIRST TEST)

Treatment	Cyto-geneticist	No. of SCEs	No. of Chromosomes	No. of Cells	Chromosomes/Cell (Mean ± SD)	SCEs/Cell (Mean ± SE)	SCEs/Chromosome (Mean ± SE)
Negative control (1% DMSO)	A	252	474	25	18.96 ± 0.84	10.08 ± 0.63	0.532 ± 0.033
	B	300	478	25	19.12 ± 0.73	12.00 ± 0.69	0.628 ± 0.036
Azinphos-methyl (μg/ml)							
5	A	--[*]	--	--	--	--	--
	B	--[*]	--	--	--	--	--
10	A	261	473	25	18.92 ± 0.64	10.44 ± 0.65	0.552 ± 0.034
	B	326	472	25	18.88 ± 0.83	13.04 ± 0.72	0.691 ± 0.038
20	A	331	481	25	19.24 ± 0.72	13.24 ± 0.73	0.688 ± 0.038
	B	104	169	9[†]	18.78 ± 1.30	11.56 ± 1.13	0.615 ± 0.060
40	A	325	476	25	19.04 ± 0.68	13.00 ± 0.72	0.683 ± 0.038
	B	282	417	22[†]	18.95 ± 1.00	12.82 ± 0.76	0.676 ± 0.040
80	A	--[‡]	--	--	--	--	--
	B	--[‡]	--	--	--	--	--
Positive control (10^{-3}M ethyl methanesulfonate)	A	880	477	25	19.08 ± 0.76	35.20 ± 1.19	1.845 ± 0.062
	B	1049	477	25	19.08 ± 0.86	41.96 ± 1.30	2.199 ± 0.068

---

[*] Cultures were not evaluated because they were lost during harvest.

[†] Insufficient M_2 metaphase cells for further analysis.

[‡] Cultures were not harvested because of excessive cytotoxicity.

Table 28

SISTER CHROMATID EXCHANGE (SCE) FREQUENCIES IN CHINESE HAMSTER OVARY (CHO) CELLS EXPOSED TO AZINPHOS-METHYL FOR 21.5 HOURS WITHOUT METABOLIC ACTIVATION (REPEAT TEST)

Treatment	Cyto-geneticist	No. of SCEs	No. of Chromosomes	No. of Cells	Chromosomes/Cell (Mean ± SD)	SCEs/Cell (Mean ± SE)	SCEs/Chromosome (Mean ± SE)
Negative control (1% DMSO)	A	278	482	25	19.28 ± 0.89	11.12 ± 0.67	0.577 ± 0.035
	B	207	483	25	19.32 ± 1.25	8.28 ± 0.58	0.429 ± 0.030
Azinphos-methyl (µg/ml)							
5	A	224	474	25	18.96 ± 1.06	8.96 ± 0.60	0.473 ± 0.032
	B	48	97	5*	19.40 ± 0.58	9.60 ± 1.39	0.495 ± 0.071
10	A	225	470	25	18.80 ± 0.76	9.00 ± 0.60	0.479 ± 0.032
	B	245	483	25	19.32 ± 1.22	9.80 ± 0.63	0.507 ± 0.032
20	A	238	475	25	19.00 ± 0.65	9.52 ± 0.62	0.501 ± 0.032
	B	317	483	25	19.37 ± 1.11	12.68 ± 0.71	0.656 ± 0.037
40	A	290	472	25	18.88 ± 0.88	11.60 ± 0.68	0.614 ± 0.036
	B	--†	--	--	--	--	--
80	A	--†	--	--	--	--	--
	B	1109	480	25	19.20 ± 0.82	44.36 ± 1.33	2.310 ± 0.069
Positive control (10^{-3} M ethyl methanesulfonate)	A	907	476	25	19.04 ± 0.84	36.28 ± 1.20	1.905 ± 0.063
	B	360	492	25	19.68 ± 0.95	14.40 ± 0.76	0.732 ± 0.039

---

[*] Due to poor contrast, this sample could not be evaluated further.

[†] This sample was not analyzed because of insufficient M_2 metaphase cells.

Table 29

SISTER CHROMATID EXCHANGE (SCE) FREQUENCIES IN CHINESE HAMSTER OVARY (CHO) CELLS EXPOSED TO AZINPHOS-METHYL FOR 2 HOURS WITH METABOLIC ACTIVATION

Treatment	Cyto-geneticist*	No. of SCEs	No. of Chromosomes	Chromosomes/Cell (Mean ± SD)	SCEs/Cell† (Mean ± SE)	SCEs/Chromosome† (Mean ± SE)
Negative control (% DMSO)	A	284	498	19.92 ± 1.47	11.36 ± 0.67	0.570 ± 0.034
	B	258	473	18.92 ± 0.64	10.32 ± 0.64	0.545 ± 0.034
Azinphos-methyl (μg/ml)						
25	A	345	488	19.52 ± 1.12	13.80 ± 0.74	0.707 ± 0.038
	B	263	482	19.28 ± 0.68	10.52 ± 0.65	0.546 ± 0.034
50	A	341	475	19.00 ± 1.15	13.64 ± 0.74	0.718 ± 0.039
	B	303	482	19.28 ± 0.84	12.12 ± 0.70	0.629 ± 0.036
100	A	410	491	19.64 ± 1.29	16.40 ± 0.81	0.835 ± 0.041
	B	354	482	19.28 ± 0.68	14.16 ± 0.75	0.734 ± 0.039
200	A	365	479	19.16 ± 0.90	14.60 ± 0.76	0.762 ± 0.040
	B	351	481	19.24 ± 0.88	14.04 ± 0.75	0.730 ± 0.039
400	A	--‡	--	--	--	--
	B	--‡	--	--	--	--
Positive control (10^{-3} M dimethylnitrosamine)	A	900	495	19.80 ± 1.12	36.00 ± 1.20	1.818 ± 0.061
	B	955	479	19.16 ± 0.90	38.20 ± 1.24	1.994 ± 0.065

---

[*] Each cytogeneticist analyzed 25 cells per sample.

[†] A one-way analysis of variance comparing the SCE frequencies in CHO cells exposed to azinphos-methyl and to the negative control indicated that the variance between treatment groups was not significantly greater than the variance within treatment groups.

[‡] These cultures were not evaluated because of excessive cytotoxicity.

Table 30

MOUSE LYMPHOMA FORWARD MUTATION CONCENTRATION-RANGE ASSAY OF AZINPHOS-METHYL IN THE ABSENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (µg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency (× 10^{-6})	Induced Mutation Frequency (× 10^{-6})
Solvent control (1% DMSO)	100.0 100.0	600 600	555 545	102 103	100.9 99.1	100.9 99.1	37 38	-- --
Positive control (500 µg/ml EMS)	69.1 69.1	600 600	326 353	1035 1135	59.3 64.2	41.0 44.3	635 643	597 605
Azinphos-methyl								
1	100.3	600	497	129	90.4	90.6	52	14
2	104.7	600	589	142	107.1	112.1	48	10
5	95.8	600	541	114	98.4	94.2	42	4
10	87.9	600	529	174	96.2	84.5	66	28
20	60.0	600	512	193	93.1	55.9	75	37
40	38.8	600	479	132	87.1	33.8	55	17
80	1.8	600	408	337	74.2	1.3	165	127
100	1.3	NC*	--	--	--	--	--	--
150	0.3	NC	--	--	--	--	--	--
200	0.9	NC	--	--	--	--	--	--
300	0.4	NC	--	--	--	--	--	--
400	0.4	NC	--	--	--	--	--	--
500	PPT†	--	--	--	--	--	--	--
600	PPT	--	--	--	--	--	--	--
700	PPT	--	--	--	--	--	--	--
800	PPT	--	--	--	--	--	--	--
900	PPT	--	--	--	--	--	--	--
1000	PPT	--	--	--	--	--	--	--

---

*Not cloned.

†Persistent precipitate present after washdown procedure; sample discarded.

Table 31

MOUSE LYMPHOMA FORWARD MUTATION CONCENTRATION-RANGE ASSAY OF AZINPHOS-METHYL IN THE PRESENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency ($\times 10^{-6}$)	Induced Mutation Frequency ($\times 10^{-6}$)
Solvent control	100.6	600	534	298	91.4	91.9	112	--
(1% DMSO)	99.4	600	635	262	108.6	108.0	83	--
Positive control	80.6	600	447	744	76.5	61.6	333	235
(5 μg/ml 3-MCA)	76.8	600	417	709	71.3	54.8	340	242
Azinphos-methyl								
1	92.8	600	477	283	81.6	75.7	119	21
2	95.1	600	451	231	77.2	73.4	102	4
5	95.1	600	500	309	85.5	81.4	124	26
10	57.2	600	519	361	88.8	50.8	139	41
20	37.8	600	473	305	80.9	30.6	129	31
40	37.3	600	536	337	91.7	34.2	126	28
80	28.0	600	544	365	93.1	26.1	134	36
100	28.2	600	450	431	77.0	21.7	192	94
150	4.2	NC*	--	--	--	--	--	--
200	1.0	NC	--	--	--	--	--	--
300	0.7	NC	--	--	--	--	--	--
400	1.2	NC	--	--	--	--	--	--
500	0.8	NC	--	--	--	--	--	--
600	PPT†	--	--	--	--	--	--	--
700	PPT	--	--	--	--	--	--	--
800	PPT	--	--	--	--	--	--	--
900	PPT	--	--	--	--	--	--	--
1000	PPT	--	--	--	--	--	--	--

---

*Not cloned.

†Persistent precipitate present after washdown procedure; sample discarded.

Table 32

MOUSE LYMPHOMA FORWARD MUTATION ASSAY OF AZINPHOS-METHYL IN THE ABSENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency ($\times 10^{-6}$)	Induced Mutation Frequency ($\times 10^{-6}$)
Solvent control (1% DMSO)	102.2	600	646	232	99.2	101.3	72	--
	97.8	600	657	179	100.8	98.6	54	--
Positive control (500 μg/ml EMS)	65.3	600	285	1090	43.7	28.6	765	702
	63.0	600	242	1209	37.1	23.4	999	936
Azinphos-methyl								
0.9	102.2	600	664	156	101.9	104.2	47	--
	102.7	600	619	166	95.0	97.6	54	--
6	97.8	600	668	143	102.5	100.3	43	--
	97.8	600	590	179	90.6	88.6	61	--
9	93.9	600	582	201	89.3	83.9	69	6
	102.7	600	700	169	107.4	110.3	48	--
15	79.5	600	602	155	92.4	73.5	51	--
	74.1	600	640	195	98.2	72.8	61	--
21	57.0	600	685	273	105.1	59.9	80	17
	58.1	600	634	211	97.3	56.5	67	4
27	50.1	600	590	196	90.6	45.4	66	3
	48.8	600	520	220	79.8	39.0	85	22
45	22.7	600	532	295	81.7	18.5	111	48
	21.9	600	564	286	86.6	19.0	101	38
54	10.0	600	526	320	80.7	8.1	122	59
	9.6	600	441	362	67.7	6.5	164	101
63	2.6	600	404	338	62.0	1.6	167	104
	2.6	600	383	382	58.8	1.5	199	136
81	2.2	NC*	--	--	--	--	--	--
	1.8	NC	--	--	--	--	--	--

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*Not cloned.

Table 33

MOUSE LYMPHOMA FORWARD MUTATION ASSAY OF AZINPHOS-METHYL IN THE PRESENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency ($\times 10^{-6}$)	Induced Mutation Frequency ($\times 10^{-6}$)
Solvent control (1% DMSO)	96.7	600	554	217	103.7	100.3	78	--
	103.3	600	514	204	96.3	99.4	79	--
Positive control (6 μg/ml 3-MCA)	46.4	600	235	374	44.0	20.4	318	239
	46.6	600	232	412	43.4	20.2	355	276
Azinphos-methyl								
6	85.7	600	398	357	74.5	63.9	179	100
	84.6	600	392	384	73.4	62.1	196	117
8	71.1	600	430	274	80.5	57.3	127	48
	84.8	600	388	301	72.7	61.6	155	76
10	54.0	600	373	318	69.9	37.7	171	92
	59.1	600	182	285	34.1	20.1	313	234
15	29.6	600	194	436	36.3	10.8	449	370
	37.4	600	152	313	28.5	10.6	412	333
42	19.1	600	204	410	38.2	7.3	402	323
	20.4	600	149	311	27.9	5.7	417	338
75	22.7	600	--*	297	--	--	--	--
	22.6	600	445	243	83.3	18.8	109	30
102	12.6	600	328	409	61.4	7.7	249	170
	12.6	600	330	413	61.8	7.8	250	171
108	10.2	600	312	368	58.4	6.0	236	157
	11.4	600	374	397	70.0	8.0	212	133
114	7.7	NC†	--	--	--	--	--	--
	7.4	NC	--	--	--	--	--	--
126	3.4	NC	--	--	--	--	--	--
	3.6	NC	--	--	--	--	--	--

---

*Sample lost.

†Not cloned.

Table 34

UNSCHEDULED DNA SYNTHESIS ASSAY OF AZINOPHOS-METHYL WITHOUT ACTIVATION

(dpm/μg DNA)
Retest 1

	Concentration of Compounds Tested						
	Azinophos-methyl (M x 10^{-5})						
Sample	0[*]	3.1	6.3	11.1	33.3	100[†]	4NQO (M) 10^{-5}
1	45	47	31	14	11	--[‡]	1295
2	54	45	42	22	14	5	1526
3	59	54	31	47	9	13	1487
4	61	43	33	24	10	8	1397
5	54	40	32	25	8	13	1418
6	59	36	46	27	12	9	561
Mean	55	44	36	26	11	9	1281
SD	6	6	6	11	2	3	362
SE	3	3	3	4	1	1	148

---

[*] Negative control and compound solvent, 0.5% DMSO.

[†] Precipitate observed at 10^{-3} M.

[‡] Sample lost.

Table 35

UNSCHEDULED DNA SYNTHESIS ASSAY OF AZINPHOS-METHYL
WITH METABOLIC ACTIVATION
(dpm/μg DNA)
Retest 1

Sample	Concentration of Compounds Tested						
	Azinphos-methyl (M x 10^{-5})						DMN (M)
	0*	3.7	11.1	33.3	100	300†	5 x 10^{-2}
1	155	188	162	154	216	--	958
2	240	179	205	319	252	--	799
3	170	173	196	204	158	--	1070
4	187	376	266	247	147	--	598
5	225	283	242	183	138	--	905
6	234	162	139	194	188	--	452
Mean	202	227	202	217	183	--	797
SD	36	85	47	59	44	--	232
SE	15	35	19	24	18	--	95

---

[*] Negative control and compound solvent, 0.5% DMSO.

[†] On reaction with Diphenylamine, these samples produced a deep purple color which made the estimation of the quantity of DNA impossible.

Table 36

UNSCHEDULED DNA SYNTHESIS ASSAY OF AZINPHOS-METHYL WITH METABOLIC ACTIVATION
(dpm/µg DNA)
Retest 2

	Concentration of Compounds Tested						
	Azinphos-methyl (M x 10^{-5})						DMN (M)
Sample	0*	1.23	3.7	11.1	33.3	100	5 x 10^{-2}
1	108	114	84	111	88	125	461
2	130	88	76	79	81	94	249
3	83	93	79	80	69	134	278
4	106	99	90	86	84	91	230
5	101	105	78	88	93	111	204
6	117	109	94	124	124	86	248
Mean	108	101	83	94	90	107	278
SD	16	10	7	19	18	20	93
SE	6	4	3	8	7	8	38

---

*Negative control and compound solvent, 0.5% DMSO.

Table 37

DOSAGE AND SACRIFICE SCHEDULE

Compound	No. Animals	0 Hr	24 Hr	Mean Body Weight Change (g)	No. Sacrificed 30 Hr	No. Sacrificed 48 Hr	No. Sacrificed 72 Hr	No. Fatalities
TMP--Positive control	9	1.25 g/kg	1.25 g/kg	-2.1	8	--	--	0
DMSO--Vehicle control	27	5 ml/kg	5 ml/kg	-0.6	8	8	8	0
Azinphos-methyl								
Low Dose	27	1 mg/kg	1 mg/kg	-0.1	8	8	8	0
Medium Dose	27	2 mg/kg	2 mg/kg	-0.8	8	8	8	0
High Dose	27	4 mg/kg	4 mg/kg	-1.5	7	7	6	7

Table 38

INCIDENCE OF MICRONUCLEATED POLYCHROMATOPHILIC ERYTHROCYTES IN BLOOD AND BONE MARROW*
(500 PCEs scored per smear)

	30 Hours				48 Hours				72 Hours			
Dose	Animal No.	Blood PCE with MN	BM PCE with MN	BM PCE/RBC	Animal No.	Blood PCE with MN	BM PCE with MN	BM PCE/RBC	Animal No.	Blood PCE with MN	BM PCE with MN	BM PCE/RBC
DMSO, 5 ml/kg	B0	1	0	0.6	C0	1	0	1.0	A0	NS	0	0.7
	B1	0	0	0.7	C1	1	2	0.5	A1	0	0	1.1
	B2	NS	0	0.8	C2	0	2	0.6	A2	NS	0	1.0
	B3	3	1	0.6	C3	0	2	1.6	A3	NS	0	0.8
	E0	0	1	0.6	D0	4	2	1.3	F0	1	1	0.7
	E1	2	1	0.8	D1	2	1	1.5	F1	3	1	1.0
	E2	0	0	0.8	D2	4	0	1.2	F2	1	0	0.7
	E3	0	1	1.9	D3	0	0	1.1	F3	1	1	0.7
	Total	6	4			12	9			--	3	
	Mean			0.9				1.1				0.8
Azinphos-methyl 1 mg/kg	K0	2	1	0.9	M0	2	0	0.9	L0	NS	1	1.1
	K1	0	1	1.1	M1	3	0	0.4	L1	1	3	0.8
	K2	2	0	0.6	M2	2	0	0.8	L2	0	1	2.0
	K3	1	0	0.6	M3	1	1	0.7	L3	NS	3	0.8
	O0	0	1	0.9	P0	0	0	2.1	N0	3	2	0.8
	O1	1	0	1.3	P1	1	0	1.1	N1	1	0	1.2
	O2	2	1	1.1	P2	1	1	1.4	N2	1	1	1.2
	O3	0	0	2.0	P3	2	0	1.5	N3	0	0	0.4
	Total	8	4			12	2			--	11	
	Mean			1.1				1.1				1.0
Azinphos-methyl 2 mg/kg	S0	2	2	1.1	R0	0	0	0.5	T0	NS	0	2.1
	S1	2	1	0.7	R1	1	0	1.1	T1	0	2	1.2
	S2	0	2	1.0	R2	1	1	0.9	T2	NS	2	1.9
	S3	0	0	0.8	R3	1	2	0.6	T3	NS	1	1.5
	U0	1	2	1.0	V0	3	0	1.4	W0	1	2	1.8
	U1	0	0	1.6	V1	0	0	0.8	W1	2	0	0.7
	U2	1	1	1.0	V2	2	1	1.4	W2	0	1	0.7
	U3	1	1	1.2	V3	1	0	1.0	W3	NS	1	0.8
	Total	7	9			9	4			--	9	
	Mean			0.9				1.0				1.3

(Continued)

Table 38 (Concluded)

	30 Hours				48 Hours				72 Hours			
Dose	Animal No.	Blood PCE with MN	BM PCE with MN	BM PCE/RBC	Animal No.	Blood PCE with MN	BM PCE with MN	BM PCE/RBC	Animal No.	Blood PCE with MN	BM PCE with MN	BM PCE/RBC
Azinphos-methyl 4 mg/kg	BB0	0	1	1.1	Y0	1	3	0.8	AA0	NS	1	1.2
	BB1	0	0	0.7	Y1	0	1	0.5	AA1	NS	1	0.8
	BB2	1	1	0.9	Y2	1	0	0.3	AA2	NS	0	2.3
	DD1	1	1	0.9	Y3	0	2	1.0	CC2	NS	1	0.2
	DD2	2	1	1.4	Z0	0	0	0.4	CC3	0	2	1.6
	DD3	0	2	1.6	Z2	0	0	1.0	EE2	0	0	0.7
	EE0	2	1	1.8	Z3	0	0	1.2				
	Total	6	7			2	6			--	5	
	Mean			1.2				0.7				1.1
TMP, 1.25 g/kg	H0	15	14	0.6								
	H1	15	4	0.6								
	H2	7	2	0.7								
	H3	10	17	0.9								
	I0	12	18	0.6								
	I1	11	12	0.7								
	I2	11	19	0.9								
	I3	5	11	0.7								
	Total	86	97									
	Mean			0.7								

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* BM = bone marrow
PCE = polychromatophilic erythrocyte
RBC = mature erythrocyte
MN = micronucleus
NS = not scoreable (usually because the smear was made too thin).

Benomyl

SCE in CHO Cells (Tables 39-44). In the cytotoxicity test with metabolic activation, benomyl was dissolved in 95% ethanol at a concentration of 181.8 mg/ml. However, in the cytotoxicity test without metabolic activation, the maximum concentration achieved in 95% ethanol was 70.35 mg/ml of benomyl (Table 39). The laboratory was noticeably colder on the day of the cytotoxicity test without metabolic activation, which may explain the lower solubility of benomyl in ethanol on that day. At a concentration of 181.8 mg/ml, benomyl caused the metabolic activation mixture to become cloudy.

Table 40 presents the results of the cytotoxicity test of benomyl in CHO cell cultures with metabolic activation. At concentrations of 363.6 µg/ml, or greater, the cultures yielded primarily M_1 metaphases. At concentrations of 72.7 µg/ml or less, M_2 metaphases were produced.

Table 41 presents the SCE frequencies in CHO cells exposed to benomyl without metabolic activation. At benomyl concentrations of 5 and 10 µg/ml, the number of M_2 metaphase cells produced was not sufficient for analysis. A one-way analysis of variance was conducted on SCE frequencies of the three lower concentrations of benomyl and of the negative controls. The variance between treatment groups was not significantly greater than the variance within treatment groups. However, there was a discrepancy between the observations of the two cytogeneticists; B's results indicated an elevation in SCE frequencies in response to benomyl (peaking at 1.25 µg/ml) while A's results did not.

Because of the discrepancy, a third cytogeneticist was asked to evaluate the slides. Table 42 presents the observations of all three cytogeneticists. Like cytogeneticist A, cytogeneticist C observed an increase in SCE frequencies in response to benomyl. However, this response appeared to plateau at the three concentrations evaluated. Statistical analysis of the results of all three cytogeneticists revealed that the variance between groups was significantly greater than the variance within groups. We interpret these results to indicate induction of SCEs in CHO cells by benomyl without metabolic activation, although the response was weak.

Table 43 present the SCE frequencies in CHO cells exposed to benomyl with metabolic activation. Because of an error in coding the slides, cytogeneticist B evaluated two sets of slides prepared from Sample 14 (positive control) and did not examine slides prepared from Sample 12 (150 µg/ml benomyl). A one-way analysis of variance was conducted on the SCE frequencies of the four lower concentrations of benomyl and of the negative controls. The variance between treatment groups was not significantly greater than the variance within treatment groups. However, the scores of both observers indicated a concentration-related elevation in SCE frequencies in response to benomyl, at least for the three lower concentrations.

Since the coding error prevented statistical consideration of SCE frequencies in cells exposed to the highest tested concentration of benomyl (replicate observations are required for an analysis of variance), and since concentration-related trend in response to benomyl was suggested, a third cytogeneticist was asked to evaluate these coded slides (including the sample exposed to 150 μg/ml benomyl that was not previously scored). Table 44 presents the observations of all three cytogeneticists. On the basis of the combined results, the response appeared to be concentration-related. For all three scorers, the variance between groups was significantly greater than the variance within groups.

For the present, we conclude that under the conditions of these experiments, benomyl induced SCEs in CHO cells with and without metabolic activation. Without activation, the response appeared to plateau at the three concentrations evaluated; with activation, a concentration-related trend was observed. Additional testing of benomyl in this system would clarify the interpretation.

L5178Y Mouse Lymphoma (Tables 45-48). Table 45 presents the results of concentration-range testing of benomyl in the absence of the metabolic activation system. The results showed that the range to use in the mutagenesis test was approximately 2 to 40 μg/ml (108.7% relative total growth at 2 μg/ml and 15.3% relative total growth at 40 μg/ml). Concentration-range test results of benomyl in the presence of the metabolic activation system (Table 46) showed that the approximate range to use in the mutagenesis assay was 5 to 100 μg/ml (115.2% relative total growth at 5 μg/ml and 14.5% relative total growth at 100 μg/ml).

Table 47 presents the results of mutagenesis testing of benomyl in the absence of the metabolic activation system. Benomyl was extremely toxic at concentrations greater than 50 μg/ml, yielding relative total growth values of less than 2.0%. The lowest concentration tested (1 μg/ml) yielded an average relative total growth of 87.5%. Over the range tested, a concentration-related increase in induced mutation frequency was observed. An average mutation frequency of at least twice the spontaneous frequency was observed at 50 μg/ml with an average relative total growth value of 21.2%.

Table 48 presents the results of mutagenesis testing of benomyl in the presence of the metabolic activation system. The highest concentration tested (25 μg/ml) yielded an average relative total growth value of 9.8%. The lowest concentration tested (5 μg/ml) yielded an average relative total growth value of 83.3%. Over the range tested, a concentration-related increase in induced mutation frequency was observed. Average mutation frequencies of at least twice the spontaneous frequency were observed from 12 to 20 μg/ml (seven concentrations), with average relative total growth values of at least 10.0%.

These combined results indicate a mutagenic effect of benomyl at the thymidine kinase locus in L5178Y mouse lymphoma cells, both with and without the metabolic activation system. In both cases benomyl increased the mutation frequency, in a concentration-related manner, above the frequencies observed with the solvent control. The presence of the metabolic activation system appeared to enhance the mutagenic effect of benomyl.

Micronucleus Test (Tables 49, 50). The dosing and sacrifice schedule is shown in Table 49. The incidences of micronuclei in PCEs and the PCE to RBC ratios are given in Table 50. The high-dose groups were positive at both 48 and 72 hours ($\alpha < 0.05$). The 48-hour group actually represents animals sacrificed 24 hours after a single dose. The 72-hour group was dosed at both 0 and 24 hours, although the actual amount administered at 0 hours was probably less than the 1 g/kg intended. The low- and medium-dose groups were positive ($\alpha < 0.05$) 48 hours and negative ($\beta < 0.05$) 72 hours after dosing at 0 and 24 hours. The vehicle-control groups were negative at both times. Although a few animals in both the treated and control groups had elevated PCE to RBC ratios, the mean ratio for each dose/time group is within normal limits ($\bar{x}$ = 0.5 to 1.5).

Because four dose/time groups are in the positive range ($\alpha \leq 0.05$), benomyl meets the criteria for mutagenesis in the mouse micronucleus test.

Table 39

CYTOTOXICITY TEST OF CHINESE HAMSTER OVARY CELLS EXPOSED TO BENOMYL FOR 21.5 HOURS WITHOUT METABOLIC ACTIVATION

Treatment	Number of Divisions in Bromodeoxyuridine (BrdU)[*]
Negative control (0.95% ethanol)	M_2
Benomyl (μg/ml)	
1.13	$M_2 > M_1$[†]
5.63	M_2
28.14	--[‡]
140.7	--[‡]
703.5	--[‡]
Positive control (10^{-3} M ethyl methanesulfonate)	M_2

---

[*] M_1, one division in BrdU; M_2, two divisions in BrdU.

[†] The cell type to the left of the > sign was in the majority.

[‡] Cells not harvested due to excessive cytotoxicity.

Table 40

CYTOTOXICITY TEST OF CHINESE HAMSTER OVARY CELLS EXPOSED TO BENOMYL FOR 2 HOURS WITH METABOLIC ACTIVATION

Treatment	Number of Divisions in Bromodeoxyuridine (BrdU)[*]
Negative control (0.95% ethanol)	M_2
Benomyl (μg/ml)	
2.9	M_2 >> M_1[†]
14.5	M_2 >> M_1[†]
72.7	M_2 >> M_1[†]
363.6	M_1 >> M_2[†]
1818	M_1
Positive control (10^{-3} M dimethylnitrosamine)	M_2

---

[*] M_1, one division in BrdU; M_2, two divisions in BrdU.

[†] The cell type to the left of the >> sign was in great excess.

Table 41

SISTER CHROMATID EXCHANGE (SCE) FREQUENCIES IN CHINESE HAMSTER OVARY (CHO) CELLS EXPOSED TO BENOMYL FOR 21.5 HOURS WITHOUT METABOLIC ACTIVATION

Treatment	Cyto-genet-icist*	No. of SCEs	No. of Chromosomes	Chromosomes/Cell (Mean ± SD)	SCEs/Cell† (Mean ± SE)	SCEs/Chromosome† (Mean ± SE)
Negative control (0.95% ethanol)	A	315	493	19.72 ± 0.46	12.60 ± 0.71	0.639 ± 0.036
	B	243	478	19.12 ± 1.13	9.72 ± 0.62	0.508 ± 0.033
Benomyl (µg/ml)						
0.625	A	350	498	19.92 ± 0.81	14.00 ± 0.75	0.703 ± 0.038
	B	343	481	19.24 ± 0.44	13.72 ± 0.74	0.713 ± 0.039
1.25	A	322	498	19.92 ± 0.91	12.88 ± 0.72	0.647 ± 0.036
	B	376	479	19.16 ± 0.69	15.04 ± 0.78	0.785 ± 0.041
2.5	A	360	496	19.84 ± 1.28	14.40 ± 0.76	0.726 ± 0.038
	B	331	468	18.72 ± 1.43	13.24 ± 0.73	0.707 ± 0.039
5	--‡	--	--	--	--	--
10	--‡	--	--	--	--	--
Positive control (10^{-3} M ethyl methanesulfonate)	A	1062	492	19.68 ± 1.07	42.48 ± 1.30	2.159 ± 0.066
	B	1144	486	19.44 ± 0.65	45.76 ± 1.35	2.354 ± 0.070

---

[*] Each cytogeneticist analyzed 25 cells per sample.

[†] A one-way analysis of variance comparing the SCE frequencies in CHO cells exposed to benomyl and to the negative control indicated that the variance between treatment groups was not significantly greater than the variance within treatment groups.

[‡] Insufficient number of M_2 metaphase cells for analysis.

Table 42

SISTER CHROMATID EXCHANGE (SCE) FREQUENCIES IN CHINESE HAMSTER OVARY (CHO) CELLS EXPOSED TO BENOMYL FOR 21.5 HOURS WITHOUT METABOLIC ACTIVATION
(3 Cytogeneticists)

Treatment	Sample No.	Cytogeneticist[*]	No. of SCEs	No. of Chromosomes	Chromosomes/Cell (Mean ± SD)	SCEs/Cell[†] (Mean ± SE)	SCEs/Chromosome[†] (Mean ± SE)
Negative control (0.95% ethanol)	1	A	315	493	19.72 ± 0.46	12.60 ± 0.71	0.639 ± 0.036
	1	C	248	487	19.48 ± 0.92	9.92 ± 0.63	0.509 ± 0.032
	2	B	243	478	19.12 ± 1.13	9.72 ± 0.62	0.508 ± 0.033
	2	C	243	495	19.80 ± 1.15	9.72 ± 0.62	0.491 ± 0.031
Benomyl (μg/ml)							
0.625	3	A	350	498	19.92 ± 0.81	14.00 ± 0.75	0.703 ± 0.038
	3	C	345	494	19.76 ± 0.88	13.80 ± 0.74	0.698 ± 0.038
	4	B	343	481	19.24 ± 0.44	13.72 ± 0.74	0.713 ± 0.039
1.25	5	A	322	498	19.92 ± 0.91	12.88 ± 0.72	0.647 ± 0.036
	5	C	337	496	19.84 ± 1.03	13.48 ± 0.73	0.679 ± 0.037
	6	B	376	479	19.16 ± 0.69	15.04 ± 0.78	0.785 ± 0.041
	6	C	337	499	19.96 ± 1.06	13.48 ± 0.73	0.675 ± 0.037
2.5	7	B	331	468	18.72 ± 1.43	13.24 ± 0.73	0.707 ± 0.039
	7	C	365	500	20.00 ± 1.29	14.60 ± 0.76	0.730 ± 0.038
	8	A	360	496	19.84 ± 1.28	14.40 ± 0.76	0.726 ± 0.038
5		‡					
10		‡					
Positive control (10^{-3} M ethyl methanesulfonate)	9	A	1062	492	19.68 ± 1.07	42.48 ± 1.30	2.159 ± 0.066
	10	B	1144	486	19.44 ± 0.65	45.76 ± 1.35	2.354 ± 0.070

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[*] Each cytogeneticist analyzed 25 cells per sample.

[†] A one-way analysis of variance comparing the SCE frequencies in CHO cells exposed to benomyl and to the negative control indicated that the variance between treatment groups was significantly greater ($P < 0.005$) than the variance within treatment groups.

[‡] Insufficient number of M_2 metaphase cells for analysis.

Table 43

SISTER CHROMATID EXCHANGE (SCE) FREQUENCIES IN CHINESE HAMSTER OVARY (CHO) CELLS

(2 Cytogeneticists)

Treatment	Sample No.	Cyto-genet-icist[*]	No. of SCEs	No. of Chromosomes	Chromosomes/Cell (Mean ± SD)	SCEs/Cell[†] (Mean ± SE)	SCEs/Chromosome[†] (Mean ± SE)
Negative control (0.95% ethanol)	1	A	420	473	18.92 ± 1.15	16.80 ± 0.81	0.888 ± 0.045
	2	B	532	496	19.84 ± 0.90	21.28 ± 0.92	1.073 ± 0.047
Benomyl (μg/ml)							
9.375	3	A	601	477	19.08 ± 1.41	24.04 ± 0.99	1.260 ± 0.051
	4	B	757	503	20.12 ± 0.83	30.28 ± 1.10	1.505 ± 0.055
18.75	5	A	640	473	18.92 ± 1.58	25.60 ± 1.01	1.353 ± 0.053
	6	B	797	494	19.76 ± 0.72	31.48 ± 1.12	1.613 ± 0.057
37.5	7	A	838	483	19.32 ± 1.25	33.52 ± 1.16	1.735 ± 0.060
	8	B	795	493	19.72 ± 0.98	31.80 ± 1.13	1.613 ± 0.057
75	9	A	652	488	19.52 ± 1.45	26.08 ± 1.02	1.336 ± 0.052
	10	B	1014	492	19.68 ± 0.85	40.56 ± 1.27	2.061 ± 0.065
150	11	A	754	475	19.01 ± 1.71	30.16 ± 1.10	1.587 ± 0.058
	12	--[‡]	--	--	--	--	--
Positive control (10^{-3} M dimethylnitrosamine)	13	A	908	483	19.32 ± 1.44	36.32 ± 1.21	1.880 ± 0.062
	14	B[§]	1022	484	19.36 ± 1.11	40.88 ± 1.28	2.132 ± 0.066
	14	B[§]	775	487	19.48 ± 1.12	31.00 ± 1.11	1.591 ± 0.057

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[*] Each cytogeneticist analyzed 25 cells per sample; 26 cells were evaluated for Sample No. 11.

[†] A one-way analysis of variance comparing the SCE frequencies in CHO cells exposed to benomyl and to the negative control indicated that the variance between treatment groups was not significantly greater than the variance within treatment groups.

[‡] This sample was not evaluated because of an error in coding the slides.

[§] The positive control sample No. 14 was evaluated twice by cytogeneticist B because of an error in coding the slides.

Table 44

SISTER CHROMATID EXCHANGE (SCE) FREQUENCIES IN CHINESE HAMSTER OVARY (CHO) CELLS EXPOSED TO BENOMYL FOR 2 HOURS WITH METABOLIC ACTIVATION (3 Cytogeneticists)

Treatment	Sample No.	Cyto-genet-icist[*]	No. of SCEs	No. of Chromosomes	Chromosomes/Cell (Mean ± SD)	SCEs/Cell[†] (Mean ± SE)	SCEs/ Chromosome[†] (Mean ± SE)
Negative control (0.95% ethanol)	1	A	420	473	18.92 ± 1.15	16.80 ± 0.81	0.888 ± 0.045
	2	B	532	496	19.84 ± 0.90	21.28 ± 0.92	1.073 ± 0.047
	2	C	534	484	19.36 ± 0.64	21.36 ± 0.92	1.103 ± 0.048
Benomyl (μg/ml)							
9.375	3	A	601	477	19.08 ± 1.41	24.04 ± 0.99	1.260 ± 0.051
	3	C	829	484	19.36 ± 0.76	33.16 ± 1.15	1.713 ± 0.059
	4	B	757	503	20.12 ± 0.83	30.28 ± 1.10	1.505 ± 0.055
18.75	5	A	640	473	18.92 ± 1.58	25.60 ± 1.01	1.353 ± 0.053
	6	B	797	494	19.76 ± 0.72	31.48 ± 1.12	1.613 ± 0.057
	6	C	797	477	19.08 ± 0.81	31.88 ± 1.13	1.671 ± 0.059
37.5	7	A	838	483	19.32 ± 1.25	33.52 ± 1.16	1.735 ± 0.060
	7	C	877	489	19.56 ± 0.82	35.08 ± 1.19	1.793 ± 0.061
	8	B	795	493	19.72 ± 0.98	31.80 ± 1.13	1.613 ± 0.057
75	9	A	652	488	19.52 ± 1.45	26.08 ± 1.02	1.336 ± 0.052
	9	C	837	482	19.28 ± 0.84	33.48 ± 1.16	1.737 ± 0.060
	10	B	1014	492	19.68 ± 0.85	40.56 ± 1.27	2.061 ± 0.065
150	11	A	754	475	19.01 ± 1.71	30.16 ± 1.10	1.587 ± 0.058
	12	C	959	473	18.92 ± 0.91	38.36 ± 1.24	2.027 ± 0.065
Positive control (10^{-3} M dimethylnitrosamine)	13	A	908	483	19.32 ± 1.44	36.32 ± 1.21	1.880 ± 0.062
	14	B[‡]	1022	484	19.36 ± 1.11	40.88 ± 1.28	2.132 ± 0.066
	14	B[‡]	775	487	19.48 ± 1.12	31.00 ± 1.11	1.591 ± 0.057

---

[*] Each cytogeneticist analyzed 25 cells per sample; 26 cells were evaluated for sample No. 11.

[†] A one-way analysis of variance comparing the SCE frequencies in CHO cells exposed to benomyl and to the negative control indicated that the variance between treatment groups was significantly greater ($P < 0.025$) than the variance within treatment groups.

[‡] The positive control sample No. 14 was evaluated twice by cytogeneticist B because of an error in coding the slides.

Table 45

MOUSE LYMPHOMA FORWARD MUTATION CONCENTRATION-RANGE ASSAY OF BENOMYL IN THE ABSENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (µg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency $(x\ 10^{-6})$	Induced Mutation Frequency $(x\ 10^{-6})$
Solvent control (1% DMSO)	105.6	600	478	--*	--	--	--	--
	94.4	600	584	105	100.0	94.4	36	--
Positive control (500 µg/ml EMS)	67.3	600	326	1020	55.8	37.6	626	590
	70.6	600	315	967	53.9	38.1	614	578
Benomyl								
1	99.6	600	584	97	100.0	99.6	33	--
2	95.5	600	665	91	113.9	108.7	27	--
5	79.6	600	539	77	92.3	73.5	29	--
10	82.7	600	517	130	88.5	73.2	50	14
20	78.7	600	552	104	94.5	74.4	38	2
40	35.5	600	251	104	43.0	15.3	83	47
80	PPT†	--	--	--	--	--	--	--
100	PPT	--	--	--	--	--	--	--
150	PPT	--	--	--	--	--	--	--
200	PPT	--	--	--	--	--	--	--
300	PPT	--	--	--	--	--	--	--
400	PPT	--	--	--	--	--	--	--
500	PPT	--	--	--	--	--	--	--
600	PPT	--	--	--	--	--	--	--
700	PPT	--	--	--	--	--	--	--
800	PPT	--	--	--	--	--	--	--
900	PPT	--	--	--	--	--	--	--
1000	PPT	--	--	--	--	--	--	--

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*Sample lost

†Persistent precipitate present after washdown procedure; sample discarded

Table 46

MOUSE LYMPHOMA FORWARD MUTATION CONCENTRATION-RANGE ASSAY OF BENOMYL IN THE PRESENCE OF INDUCED METABOLIC ACTIVATION

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency ($\times 10^{-6}$)	Induced Mutation Frequency ($\times 10^{-6}$)
Solvent control (1% DMSO)	95.0	600	443	152	80.3	76.2	69	--
	105.0	600	661	153	119.7	125.7	46	--
Positive control (5 μg/ml 3-MCA)	78.9	600	469	670	85.0	67.0	286	228
	85.0	600	408	642	73.9	62.8	315	257
Benomyl								
1	117.9	600	568	191	102.9	121.3	67	9
2	110.8	600	527	182	95.5	105.8	69	11
5	114.4	600	556	160	100.7	115.2	58	--
10	99.3	600	538	275	97.5	96.8	102	44
20	49.9	600	489	544	88.6	44.2	222	164
40	10.8	600	261	414	47.3	5.1	317	259
80	18.0	600	292	418	52.9	9.5	286	228
100	24.7	600	323	447	58.5	14.5	277	219
150	PPT[†]	--	--	--	--	--	--	--
200	PPT	--	--	--	--	--	--	--
300	PPT	--	--	--	--	--	--	--
400	PPT	--	--	--	--	--	--	--
500	PPT	--	--	--	--	--	--	--
600	PPT	--	--	--	--	--	--	--
700	PPT	--	--	--	--	--	--	--
800	PPT	--	--	--	--	--	--	--
900	PPT	--	--	--	--	--	--	--
1000	PPT	--	--	--	--	--	--	--

---

[*] Persistent precipitate present after washdown procedure; sample discarded.

Table 47

MOUSE LYMPHOMA FORWARD MUTATION ASSAY OF BENOMYL IN THE ABSENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency (x 10^{-6})	Induced Mutation Frequency (x 10^{-6})
Solvent control (1% DMSO)	104.0	600	491	94	103.0	107.2	38	--
	96.0	600	462	85	97.0	93.1	37	--
Positive control (500 μg/ml EMS)	58.2	600	288	943	60.4	35.2	655	617
	59.8	600	384	862	80.6	48.2	449	411
Benomyl								
1	80.1	600	542	155	113.7	91.1	57	19
	74.9	600	533	123	111.9	83.8	46	8
5	45.8	600	524	117	110.0	50.4	45	7
	48.4	600	628	140	131.8	63.8	45	7
20	44.9	600	641	124	134.5	60.4	39	1
	46.9	600	547	117	114.8	53.8	43	5
25	45.8	600	608	184	127.6	58.4	61	23
	43.8	600	583	128	122.4	53.6	44	6
30	47.3	600	490	112	102.8	48.5	46	8
	47.3	600	588	--*	123.4	58.4	--	--
35	36.7	600	514	207	107.9	39.6	81	43
	35.1	600	534	158	112.1	39.3	59	21
40	44.3	600	503	144	105.6	46.8	57	19
	47.7	600	490	150	102.8	49.1	61	23
50	32.3	600	278	169	58.3	18.8	122	84
	27.5	600	409	184	85.8	23.6	90	52
75	5.5	600	146	89	30.6	1.7	122	84
	7.4	600	70	55	14.7	1.1	157	119
100	5.1	600	51	54	10.7	0.5	212	174
	5.7	600	120	33	25.2	1.4	55	17

---

*Sample lost

Table 48

MOUSE LYMPHOMA FORWARD MUTATION ASSAY OF BENOMYL IN THE PRESENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency ($\times 10^{-6}$)	Induced Mutation Frequency ($\times 10^{-6}$)
Solvent control (1% DMSO)	99.3	600	592	195	104.1	103.4	66	--
	100.7	600	545	248	95.9	96.5	91	--
Positive control (6 μg/ml 3-MCA)	71.9	600	449	709	79.0	56.8	316	237
	72.8	600	455	778	80.0	58.3	342	263
Benomyl								
5	92.99	600	503	320	88.5	82.2	127	48
	96.6	600	496	332	87.2	84.3	134	55
10	96.6	600	489	357	86.0	83.1	146	67
	94.2	600	548	421	96.4	90.8	154	75
12	96.6	600	503	451	88.5	88.5	179	100
	94.2	600	554	432	97.4	91.8	156	77
14	91.4	600	490	530	86.2	78.8	216	137
	94.2	600	473	453	83.2	78.4	192	113
15	89.1	600	550	488	96.7	86.2	177	98
	89.1	600	494	513	86.9	77.4	208	129
16	89.1	600	465	505	81.8	72.9	217	138
	85.8	600	567	532	99.7	85.6	188	109
17	72.1	600	510	627	89.7	64.7	246	161
	85.0	600	531	490	93.4	79.4	185	106
18	76.0	600	532	595	93.6	71.1	224	145
	78.9	600	474	552	83.4	65.8	233	154
20	67.5	600	540	624	95.0	64.1	231	152
	42.2	600	385	801	67.7	28.6	416	337
25	18.3	600	283	751	49.8	9.1	531	452
	22.4	600	266	776	46.8	10.5	583	504

Table 49

DOSING AND SACRIFICE SCHEDULE

Compound	No. Animals	Dose at		Mean Body Wt Change (g)	Number Sacrificed			No. Fatalities
		0 Hr	24 Hr		48 Hr	72 Hr	96 Hr	
DMSO	24	5 ml/kg	5 ml/kg	+ 0.4	6	7	7	4
Benomyl								
Low dose	24	250 mg/kg	250 mg/kg	+ 0.4	7	8	8	1
Medium dose	24	500 mg/kg	500 mg/kg	+ 0.9	8	7	7	3
High dose	8	1000 mg/kg	1000 mg/kg	+ 0.5	-	7	-	1
	16	--	1000* mg/kg		7	-	8	1

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*In DMSO (10 ml/kg)

Table 50

INCIDENCE OF MICRONUCLEATED POLYCHROMATOPHILIC ERYTHROCYTES*
(500 PCEs Scored per Animal)

	48 Hours			72 Hours		
Dose	Animal No.	PCEs with MN	PCE/RBC	Animal No.	PCEs with MN	PCE/RBC
Vehicle control (DMSO, 5 ml/kg)	2ø	1	0.99	1ø	1	1.36
	2r	1	0.93	1r	0	1.43
	2ℓ	0	0.78	1ℓ	0	1.14
	2b	0	1.41	1b	2	1.26
	5r	2	2.08	4ø	0	0.85
	5ℓ	1	0.64	4r	1	1.69
				4b	2	1.47
	Total	5			6	
	Mean		1.14			1.31
Benomyl 2 x 250 mg/kg	7ø	1	1.19	9ø	0	0.86
	7ℓ	2	0.78	9r	1	1.12
	7b	1	0.99	9ℓ	1	1.04
	11ø	2	1.39	9b	1	1.23
	11r	5	1.13	10ø	1	1.69
	11ℓ	3	0.89	10r	1	1.32
	11b	1	1.96	10ℓ	1	1.34
				10b	0	1.28
	Total	15			6	
	Mean		1.19			1.24
Benomyl 2 x 500 mg/kg	14ø	1	1.67	15ø	2	2.35
	14r	4	1.48	15r	0	1.74
	14ℓ	2	1.56	15ℓ	0	1.54
	14b	1	1.53	15b	4	0.84
	17ø	3	0.73	18ø	1	1.06
	17r	2	1.39	18ℓ	0	1.87
	17ℓ	2	1.14	18b	0	1.24
	17b	1	0.83			
	Total	16			7	
	Mean		1.29			1.52
Benomyl 1 x 1000 mg/kg (48 hour group)	24ø	1	1.25	20ø	1	0.77
	24r	5	1.09	20r	5	0.79
	24b	4	0.59	20ℓ	7	2.94
2 x 1000 mg/kg (72 hour group)	22ø	1	1.42	19ø	1	1.16
	22r	3	1.14	19r	1	1.00
	22ℓ	3	0.93	19ℓ	2	0.78
	22b	3	1.20	19b	0	1.22
	Total	20			17	
	Mean		1.09			1.24

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*PCE = polychromatophilic erythrocytes
RBC = mature erythrocytes
MN = micronuclei

Botran

S. typhimurium Plate Incorporation (Tables 51, 52). Botran was tested once over a dose range of 10 to 5,000 µg/plate and retested at 50 to 10,000 µg/plate. Toxicity, denoted by the appearance of pinpoint colonies and/or a thinning of the background lawn, was observed at 10,000 µg/plate with all strains except TA1537. No reproducible, dose-related increase in the number of revertants was observed.

E. coli WP-2 (Tables 53, 54). Botran was tested twice over a dose range of 10 to 5,000 µg/plate. Toxicity was observed at 5,000 µg/plate with and without metabolic activation. Botran did not elicit a reproducible, dose-related increase in the number of revertants, either with or without activation.

S. cerevisiae D3 (Tables 55, 56). No recombinogenic activity was observed in the S. cerevisiae D3 assays with botran. The compound was tested once at concentrations ranging from 0.001 to 0.1% and retested over a range of 0.005 to 0.05%. Toxicity was observed at 0.05% without metabolic activation and at 0.1% with metabolic activation.

E. coli/B. subtilis Relative Toxicity (Tables 57, 58). Initially, 1.0 and 5.0 mg of botran, dissolved in DMSO, were placed on a disc. Because the samples were inclined to precipitate and remain on the disc, lower doses of 0.5 and 1.0 mg were chosen for Experiment 2. At these doses, an extract was able to move off the disc and into the agar. Botran generally demonstrated equal zones of toxicity to both repair-proficient and repair-deficient strains of E. coli and B. subtilis, indicating that botran does not cause DNA damage as detected by these procedures.

Table 51

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

BOTRAN

Experiment 1

Compound	Metabolic Activation	Amount of Compound Added/Plate	Histidine Revertants per Plate									
			TA1535		TA1537		TA1538		TA98		TA100	
Negative Control												
DMSO	-	50.0 µl	18	17	9	7	13	15	19	21	87	96
	+	50.0	19	10	25	21	21	26	36	43	96	88
Positive Controls												
Sodium Azide	-	0.5 µg	292	260								
	-	1.0									481	564
9-Aminoacridine	-	50.0			218	247						
2-Nitrofluorene	-	5.0					733	749	341	378		
2-Anthramine	-	1.0					13	18	15	28	115	113
	+	1.0					409	374	349	328	422	579
	-	2.5	28	19	15	15						
	+	2.5	261	288	152	196						
Botran	-	10.0 µg	24	20	13	19	25	19	22	20	117	117
	-	50.0	29	27	17	17	26	26	25	21	109	118
	-	100.0	32	21	16	10	25	18	24	24	114	124
	-	500.0	36	43	25	12	33	29	23	24	124	118
	-	1,000.0	21	30	12	14	34	36	25	25	116	101
	-	5,000.0	16	14	14	14	39	38	39	34	101	90
	+	10.0	17	17	36	32	43	37	41	45	141	135
	+	50.0	21	17	21	37	36	30	49	47	135	113
	+	100.0	14	24	24	17	38	26	53	33	132	120
	+	500.0	18	15	29	24	30	45	43	46	98	128
	+	1,000.0	17	20	25	20	36	41	35	41	128	128
	+	5,000.0	10	7	17	11	40	44	49	49	103	112

Table 52

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

BOTRAN

Experiment 2

Compound	Metabolic Activation	Amount of Compound Added/Plate	Histidine Revertants per Plate TA1535		TA1537		TA1538		TA98		TA100	
Negative Control												
DMSO	-	50.0 µl	29	38	16	20	18	19	30	42	129	98
	+	50.0	18	26	41	33	40	39	47	52	112	126
Positive Controls												
Sodium Azide	-	0.5 µg	246	253								
	-	1.0									340	368
9-Aminoacridine	-	50.0			142	265						
2-Nitrofluorene	-	5.0					635	630	420	434		
2-Anthramine	-	1.0					21	17	26	28	124	97
	+	1.0					405	380	261	285	548	533
	-	2.5	48	41	22	16						
	+	2.5	305	301	272	259						
Botran	-	50.0 µg	29	45	11	25	28	24	49	43	142	134
	-	100.0	36	37	13	17	18	25	32	37	143	149
	-	500.0	26	43	14	13	32	26	46	42	134	130
	-	1,000.0	28	37	18	14	37	37	41	38	109	111
	-	5,000.0	15	14	19	12	33	50	41	49	154	135
	-	10,000.0	PP*	PP	8	9	PP	PP	PP	PP	153	111
	+	50.0 µg	26	26	43	45	53	57	63	56	92	100
	+	100.0	19	21	31	41	53	61	68	65	139	111
	+	500.0	13	21	20	27	62	57	63	57	116	90
	+	1,000.0	7	17	18	30	61	65	43	65	96	104
	+	5,000.0	15	15	22	23	45	46	46	48	106	109
	+	10,000.0	PP	PP	25	15	41	43	PP	PP	PP	PP

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*PP = pinpoint colonies on background.

Table 53

IN VITRO ASSAYS WITH ESCHERICHIA COLI WP2

BOTRAN

Experiment 1

Compound	Metabolic Activation	Compound Added per Plate (µg)	Tryptophan Revertants per Plate	
Negative Control				
DMSO	-		20	23
	+		56	49
Positive Controls				
2-Anthramine	-	10.0	26	23
	+	10.0	175	186
AF-2	-	0.1	555	632
Botran	-	10.0	17	28
	-	50.0	C[*]	29
	-	100.0	23	24
	-	500.0	21	15
	-	1,000.0	14	19
	-	5,000.0	PP[†]	PP[†]
	+	10.0	22	28
	+	50.0	21	15
	+	100.0	31	27
	+	500.0	32	16
	+	1,000.0	17	27
	+	5,000.0	PP[†]	PP[†]

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[*] C = Contamination

[†] PP = Pinpoint colonies

Table 54

IN VITRO ASSAYS WITH ESCHERICHIA COLI WP2

BOTRAN

Experiment 2

Compound	Metabolic Activation	Compound Added per Plate (µg)	Tryptophan Revertants per Plate	
Negative Control DMSO	-		40	37
	+		39	46
Positive Controls				
2-Anthramine	-	10.0	36	44
	+	10.0	725	800
AF-2	-	0.1	625	646
Botran	-	10.0	33	38
	-	50.0	27	20
	-	100.0	21	17
	-	500.0	19	25
	-	1,000.0	15	14
	-	5,000.0	PP*	PP
	+	10.0	48	49
	+	50.0	41	41
	+	100.0	20	27
	+	500.0	19	19
	+	1,000.0	16	20
	+	5,000.0	PP	PP

---

*PP = pinpoint colonies.

Table 55

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
BOTRAN
Experiment 1

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors Cells per ml (x 10^{-7})	 Percent	Mitotic Recombinants Per ml (x 10^{-3})	 Per 10^5 Survivors
Negative Control						
DMSO	-		7.0	100	6.0	8.6
	+		6.7	100	6.5	10
Positive Controls						
1,2,3,4-Diepoxybutane	-	0.025	4.0	57	750	1900
	+	0.025	3.1	46	800	2600
Botran	-	0.001	7.2	103	5.0	6.9
	-	0.005	6.1	87	9.0	15
	-	0.01	6.2	89	7.0	1.2
	-	0.05	T*		T	T
	-	0.1	T		T	T
	+	0.001	7.2	107	9.0	13
	+	0.005	8.0	119	11	14
	+	0.01	4.9	73	4.0	8.2
	+	0.05	4.0	60	19	48
	+	0.1	2.9	43	10	34

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*T = toxic.

Table 56

IN VITRO ASSAYS WITH *SACCHAROMYCES CEREVISIAE* D3
BOTRAN
Experiment 2

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml (x 10^{-7})	Percent	Per ml (x 10^{-3})	Per 10^5 Survivors
Negative Control						
DMSO	-		8.2	100	7.5	9.2
	+		8.0	100	6.0	7.2
Positive Controls						
1,2,3,4-Diepoxybutane	-	0.025	6.6	80	1100	1700
	+	0.025	7.0	88	1100	1600
Botran	-	0.005	7.6	93	3.0	3.9
	-	0.0075	7.6	93	5.0	6.6
	-	0.01	7.0	85	4.0	5.7
	-	0.025	6.4	78	3.0	4.7
	-	0.05	4.2	51	1.0	2.4
	+	0.005	6.5	81	6.0	9.2
	+	0.0075	5.6	70	5.0	8.9
	+	0.01	6.4	80	2.0	3.1
	+	0.025	7.2	90	3.0	4.2
	+	0.05	5.9	74	1.0	1.7

Table 57

QUALITATIVE DIFFERENTIAL TOXICITY ASSAY

BOTRAN
Experiment 1

		Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		E. coli				B. subtilis			
Compound	Amount of Compound Added per Plate	W3110 (polA$^+$)		p3478 (polA$^-$)		H17 (rec$^+$)		M45 (rec$^-$)	
Negative Controls									
Ampicillin	20 μg	21	20	20	21				
Chloramphenicol	20					31	31	29	30
Positive Controls									
Methylmethanesulfonate	1 mg	33	30	38	36	16	15	20	20
Botran	1 mg	8	8	9	8	11	10	10	10
	5	9	9	10	10	10	9	8	9

Table 58

QUALITATIVE DIFFERENTIAL TOXICITY ASSAY

BOTRAN
Experiment 2

		Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		E. coli				B. subtilis			
Compound	Amount of Compound Added per Plate	W3110 (polA$^+$)		p3478 (polA$^-$)		H17 (rec$^+$)		M45 (rec$^-$)	
Negative Control									
Chloramphenicol	20.0 µg	25	25	25	24	30	30	30	29
Positive Control									
Methylmethanesulfonate	1.0 mg	13	14	32	26	13	13	20	17
Botran	0.5 mg	9	9	8	9	8	8	8	7
	1.0 mg	10	9	8	8	8	9	8	8

Bromacil

S. typhimurium Plate Incorporation, E. coli WP-2, S. cerevisiae D3. Bromacil had been previously tested and found to be negative in these three systems, both with and without activation (51).

S. cerevisiae D7 (Tables 59-61). Bromacil was tested over concentrations ranging from 0.05 to 2.0% in the first experiment, and from 0.025 to 1.0% in the second and third experiments. At a concentration of 0.5%, a reproducible drop in survival was observed. However, it appeared that this decrease could have been related to the relative inslubility of the compound, which might have interfered with recovery of viable cells. This potential problem was rectified in Experiment 3, in which use of longer mixing times and larger pipettes improved the recovery of surviving cells. The results from these three experiments indicate that no genetic damage was detected by the D7 strain.

E. coli/B. subtilis Relative Toxicity. Bromacil was previously tested and found negative in this assay (51).

S. typhimurium Relative Toxicity (Tables 62, 63). This compound was negative in both strain pairs in this assay.

SCE in CHO Cells (Tables 64-67). Bromacil dissolved in 100% ethanol at a maximum concentration of 125 mg/ml; however, at this concentration the solution immediately separated into two layers and it was necessary to vortex it immediately before making further dilutions. This did not occur at lower concentrations of bromacil.

Table 64 presents the results of the cytotoxicity test of bromacil in CHO cell cultures without metabolic activation. Cytotoxicity was not observed; at a concentration of 1250 μg/ml or less, M_2 metaphase cells were present. Table 65 presents the results of the cytotoxicity test of bromacil in CHO cell cultures with metabolic activation. At a concentration of 1250 μg/ml bromacil or less, the cultures yielded M_2 metaphase cells.

Table 66 presents the SCE frequencies in CHO cells exposed to bromacil without metabolic activation. Cell cultures exposed to 500 μg/ml could not be harvested because of excessive cytotoxicity. Cell cultures exposed to 250 μg/ml bromacil could not be evaluated because there were insufficient M_2 metaphase cells for analysis. A one-way analysis of variance was conducted on SCE frequencies of the other three concentrations of bromacil and of the negative controls. The variance between treatment groups was not significantly greater than the variance within treatment groups.

Table 67 presents the SCE frequencies in CHO cells exposed to bromacil with metabolic activation. At a concentration of 1250 μg/ml bromacil, the slides were not evaluated because there were insufficient M_2 metaphase cells for analysis. A one-way analysis of variance was conducted on SCE frequencies for the four lower concentrations of bromacil and of the negative controls. The variance between treatment groups was not significantly greater than the variance within treatment groups.

We conclude that bromacil did not induce SCEs in CHO cells, either with or without metabolic activation.

L5178Y Mouse Lymphoma (Tables 68-71). Table 68 presents the results of concentration-range testing of bromacil in the absence of the metabolic activation system. The results showed that the approximate range to use in the mutagenesis assay was 400 to 900 µg/ml (96.3% relative total growth at 400 µg/ml and 8.1% relative total growth at 900 µg/ml). Concentration-range test results for bromacil in the presence of the metabolic activation system (Table 69) showed that the approximate range to use in the mutagenesis assay was 5 to 40 µg/ml (110.2% relative total growth at 5 µg/ml and 15.8% relative total growth at 40 µg/ml).

Table 70 presents the results of mutagenesis testing of bromacil in the absence of the metabolic activation system. Bromacil was extremely toxic at concentrations greater than 950 µg/ml, with relative suspension growth never rising above 1.5%. No significant depression in relative growth was observed at 150 µg/ml, which yielded an average relative total growth value of 103.5%. From approximately 150 to 880 µg/ml, a concentration-related increase in induced mutation frequency was observed. Average mutation frequencies of at least twice the spontaneous frequency were observed at 750 and 850 µg/ml (the two highest concentrations with average relative total growth values of at least 10.0%).

Table 71 presents the results of mutagenesis testing of bromacil in the presence of the metabolic activation system. The highest concentration of 90 µg/ml yielded an average relative total growth value of 10.6%. No significant depression in relative total growth was observed at 35 µg/ml which yielded an average relative total growth value of 102.1%. Over the range tested, a concentration-related increase in induced mutation frequency was observed. Average mutation frequencies of at least twice the spontaneous frequency were observed from 45 to 90 µg/ml (five concentrations), with average relative total growth value of at least 10.0%.

These combined results indicate a mutagenic effect of bromacil at the thymidine kinase locus in L5178Y mouse lymphoma cells, both with and without the metabolic activation system. In both cases, bromacil increased the mutation frequency, in a concentration-related manner, above the spontaneous frequencies observed in the solvent controls. The presence of the metabolic activation system appeared to enhance the mutagenic and cytotoxic effects of bromacil.

UDS in WI-38 Cells. Bromacil was previously tested in this system and was found to be negative, both with and without activation (51).

Micronucleus Test (Tables 72, 73). The dosage and sacrifice schedules are shown in Table 72. The incidences of micronucleated PCEs in blood and bone marrow and the ratio of PCEs to mature red cells (RBCs) are given in Table 73. The bone-marrow results for all vehicle control and bromacil-treated groups were negative ($\beta < 0.005$); the positive control TMP-treated groups were positive ($\alpha < 0.001$). The mean ratio of PCEs to RBCs was within the historical control range (0.5-1.5) for all dose-time groups.

The blood values obtained in negative control and treated groups result in dose/time group decisions comparable to those based on bone marrow evaluation. The positive control groups at 30 and 48 hours had significantly fewer micronucleated cells than appeared in bone marrow samples from the same animals. At 72 hours the results in bone marrow and blood smears were the same. The vehicle control and compound-treated group totals were within the normal range for micronucleated cells in bone marrow, and the 30- and 72-hour TMP groups were clearly positive. The negative result obtained at 48 hours in blood is thought to be a chance anomaly.

The dose-related weight loss observed 24 hours after the first dose, along with the fatalities in the medium and high-dose groups, indicates that a maximum tolerated dose was administered. In this micronucleus test, a sufficient number of cells and time points were examined at each dose level, the maximum possible dose was given, and the PCE-RBC ratio was not depressed. The results for all treatment groups were negative. Bromacil is therefore negative in the micronucleus test.

Mouse Dominant Lethal Test. Bromacil was previously tested and found negative in this system (51).

Table 59

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D7

BROMACIL

Experiment 1

Compound	MA	% Conc.	Survivors: Cells Per ml ($\times 10^{-7}$)	Survivors: %	Crossing-Over: Mitotic Recombinants: Per ml ($\times 10^{-3}$)	Crossing-Over: Mitotic Recombinants: Per 10^5 Survivors	Crossing-Over: Total Aberrants: Per ml ($\times 10^{-3}$)	Crossing-Over: Total Aberrants: Per 10^5 Survivors	Gene Conversion: Convertants: Per ml ($\times 10^{-1}$)	Gene Conversion: Convertants: Per 10^6 Survivors	Reverse Mutation: Revertants: Per ml ($\times 10^{0}$)	Reverse Mutation: Revertants: Per 10^7 Survivors
Negative Control												
DMSO	-		6.1	100	1.5	2.5	4.5	7.4	76	12	11	1.8
	+		4.8	100	0	0	0	0	88	18	7.5	1.6
Positive Controls												
1,2,3,4-Diepoxybutane	-	0.006	5.1	84	110	220	400	780	1,400	270	280	55
	+	0.006	5.6	120	98	180	410	730	1,000	180	210	38
Bromacil	-	0.05	5.4	89	2.0	3.7	17	31	76	14	7.0	1.3
	-	0.1	5.3	87	4.0	7.5	24	45	94	18	10	1.9
	-	0.5	1.6	26	1.0	6.3	8.0	50	19	12	5.0	3.1
	-	1.0	2.7	44	2.0	7.4	9.0	33	29	11	7.0	2.6
	-	2.0	1.4	23	0	0	15	110	24	17	7.0	5.0
	+	0.05	5.5	110	7.0	13	40	73	80	15	8.0	1.5
	+	0.1	5.5	110	4.0	7.3	[illegible]	36	88	16	9.0	1.6
	+	0.5	3.1	65	2.0	6.5	[illegible]	55	58	19	5.0	1.6
	+	1.0	2.5	52	1.0	4.0	12	48	29	12	2.0	0.8
	+	2.0	0.3	6.0	0	0	3.0	100	2.0	6.7	6.0	20

Table 60

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D7

BROMACIL

Experiment 2

Compound	MA	% Conc.	Survivors: Cells Per ml ($\times 10^{-7}$)	Survivors: %	Crossing-Over: Mitotic Recombinants: Per ml ($\times 10^{-3}$)	Crossing-Over: Mitotic Recombinants: Per 10^5 Survivors	Crossing-Over: Total Aberrants: Per ml ($\times 10^{-3}$)	Crossing-Over: Total Aberrants: Per 10^5 Survivors	Gene Conversion: Convertants: Per ml ($\times 10^{-1}$)	Gene Conversion: Convertants: Per 10^6 Survivors	Reverse Mutation: Revertants: Per ml ($\times 10^{0}$)	Reverse Mutation: Revertants: Per 10^7 Survivors
Negative Control												
DMSO	-		4.4	100	2.5	5.7	12	27	70	16	19	4.3
	+		4.5	100	3.0	6.7	17	38	74	16	18	4.0
Positive Controls												
1,2,3,4-Diepoxybutane	-	0.006	3.4	77	78	230	580	1,700	1,300	380	530	160
	+	0.006	3.8	84	120	320	720	1,900	1,400	370	490	130
Bromacil	-	0.025	3.8	86	0	0	16	42	74	19	22	5.8
	-	0.05	3.7	84	1.0	2.7	18	49	81	22	23	6.2
	-	0.1	3.4	77	3.0	8.8	11	32	43	13	22	6.5
	-	0.5	1.6	36	3.0	19	19	120	22	14	7.0	4.4
	-	1.0	2.4	55	2.5	10	10	42	27	11	9.0	3.8
	+	0.025	3.9	87	0	0	25	6.4	83	21	18	4.6
	+	0.05	3.6	80	0	0	18	50	74	21	13	3.6
	+	0.1	3.0	67	1.0	3.3	17	57	52	17	11	3.7
	+	0.5	1.0	22	5.0	50	16	160	33	33	4.0	4.0
	+	1.0	1.7	38	3.0	18	15	88	38	22	6.0	3.5

Table 61

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D7

BROMACIL

Experiment 3

			Survivors		Crossing-Over				Gene Conversion		Reverse Mutation	
					Mitotic Recombinants		Total Aberrants		Convertants		Revertants	
Compound	MA	% Conc.	Cells Per ml ($\times 10^{-7}$)	%	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors	Per ml ($\times 10^{-1}$)	Per 10^6 Survivors	Per ml ($\times 10^0$)	Per 10^7 Survivors
Negative Control												
DMSO	-		5.4	100	2.0	3.7	17	32	72	13	16	3.0
	+		6.1	100	1.0	1.6	10	16	57	9.3	11	1.8
Positive Control												
1,2,3,4-Diepoxybutane	-	0.006	4.7	87	59	130	460	980	1,200	260	110	23
	+	0.006	4.8	79	74	150	490	1,000	1,200	250	190	40
Bromacil	-	0.025	5.1	94	0	0	18	35	74	15	12	2.4
	-	0.05	5.7	110	0	0	21	37	54	9.5	13	2.3
	-	0.1	4.4	81	0	0	7.0	16	70	16	16	3.6
	-	0.5	4.0	74	1.0	2.5	14	35	42	11	9.0	2.3
	-	1.0	5.6	100	0	0	14	25	50	8.9	12	2.1
	+	0.025	6.6	110	0	0	8.0	12	61	9.2	8.0	1.2
	+	0.05	5.9	97	0	0	17	29	86	15	13	2.2
	+	0.1	5.3	87	0	0	9.0	17	52	9.8	15	2.8
	+	0.5	5.7	93	1.0	1.8	12	21	62	11	11	19
	+	1.0	5.5	90	0	0	12	22	51	9.3	8.0	1.5

Table 62

DIFFERENTIAL TOXICITY ASSAYS WITH SALMONELLA TYPHIMURIUM STRAINS

Experiment 1

Compound	Amount of Compound Applied to Disc	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		SL4525 (rec$^+$)		SL4700 (rec$^-$)		TA1978 (uvrB$^+$)		TA1538 (uvrB$^-$0	
Negative Control									
Kanamycin	100 µg	15	16	15	15	14	15	15	16
Positive Controls									
MMS[*]	10 mg	53	53	70	70				
4-NQO[†]						12	12	24	25
Bromacil	1 mg	13	15	13	15	12	10	11	11
	5 mg	16	16	15	15	11	11	12	12

---

[*] MMS = Methylmethanesulfonate.

[†] 4-NQO = 4-Nitroquinoline-1-oxide.

Table 63

DIFFERENTIAL TOXICITY ASSAYS WITH SALMONELLA TYPHIMURIUM STRAINS

Experiment 2

Compound	Amount of Compound Applied to Disc	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		SL4525 (rec$^+$)		SL4700 (rec$^-$)		TA1978 (uvrB$^+$)		TA1538 (uvrB$^-$)	
Negative Control									
Kanamycin	100 μg	15	14	16	15	17	17	15	16
Positive Controls									
MMS*	10 mg	45	40	72	74				
4-NQO†	2 mg					12	12	28	26
Bromacil	1 mg	13	13	14	14	10	10	12	11
	5 mg	15	15	15	14	10	10	12	12

---

*MMS = Methylmethanesulfonate

†4-NQO = 4-Nitroquinoline-1-oxide.

Table 64

CYTOTOXICITY TEST OF CHINESE HAMSTER OVARY CELLS EXPOSED TO BROMACIL FOR 21.5 HOURS WITHOUT METABOLIC ACTIVATION

Treatment	Number of Divisions in Bromodeoxyuridine (BrdU)[*]
Negative Control (1% EtOH)	$M_2 > M_1$[†]
Bromacil (μg/ml)	
2	M_2
10	M_2
50	M_2
250	M_2
1250	--[‡]
Positive control (10^{-3} M ethyl methanesulfonate)	M_2

---

[*] M_1, one division in BrdU; M_2, two divisions in BrdU.

[†] The cell type to the left of the > sign was in excess.

[‡] Culture was not harvested because of excessive cytotoxicity.

Table 65

CYTOTOXICITY TEST OF CHINESE HAMSTER OVARY CELLS EXPOSED TO BROMACIL FOR 2 HOURS WITH METABOLIC ACTIVATION

Treatment	Number of Divisions in Bromodeoxyuridine (BrdU)[*]
Negative control (1% EtOH)	M_2
Bromacil (μg/ml)	M_2
2	M_2
10	M_2
50	M_2
250	M_2
1250	M_2
Positive control (10^{-3} M dimethylnitrosamine)	$M_1 + M_2$[†]

---

[*] M_1, one division in BrdU M_2, two divisions in BrdU.

[†] M_1 and M_2 cells were present.

TABLE 66

SISTER CHROMATID EXCHANGE (SCE) FREQUENCIES IN CHINESE HAMSTER OVARY (CHO) CELLS EXPOSED TO EROMACIL FOR 21.5 HOURS WITHOUT METABOLIC ACTIVATION

Treatment	Cyto-geneticist*	No. of SCEs	No. of Chromosomes	Chromosomes/Cell (Mean ± SD)	SCEs/Cell† (Mean ± SE)	SCEs/Chromosome† (Mean ± SE)
Negative control (1% Ethanol)	A	208	498	19.92 ± 0.57	8.32 ± 0.58	0.418 ± 0.029
	B	175	478	19.12 ± 0.83	7.00 ± 0.53	0.366 ± 0.028
Bromacil						
31.25 μg/ml	A	275	483	19.32 ± 0.85	11.00 ± 0.66	0.569 ± 0.034
	B	226	494	19.76 ± 0.93	9.04 ± 0.60	0.457 ± 0.030
62.5 μg/ml	A	247	494	19.76 ± 0.66	9.88 ± 0.63	0.500 ± 0.032
	B	205	484	19.36 ± 1.15	8.20 ± 0.57	0.424 ± 0.030
125 μg/ml	A	234	475	19.00 ± 1.00	9.36 ± 0.61	0.493 ± 0.032
	B	212	470	18.80 ± 0.91	8.48 ± 0.58	0.451 ± 0.031
250 μg/ml	‡	--	--	--	--	--
	‡	--	--	--	--	--
500 μg/ml	§	--	--	--	--	--
	§	--	--	--	--	--
Positive control (10^{-3} M ethyl methanesulfonate)	A	962	479	19.16 ± 0.80	38.48 ± 1.24	2.008 ± 0.065
	B	681	478	19.12 ± 1.24	27.24 ± 1.04	1.425 ± 0.055

---

[*] Each cytogeneticist analyzed 25 cells per sample.

[†] A one-way analysis of variance comparing the SCE frequenices in CHO cells exposed to Bromacil and to the negative control indicated that the variance between treatment groups was not significantly greater than the variance within treatment groups.

[‡] Insufficient M_2 metaphase cells for analysis.

[§] Cultures were not harvested because of excessive cytotoxicity.

TABLE 67

SISTER CHROMATID EXCHANGE (SCE) FREQUENCIES IN CHINESE HAMSTER OVARY (CHO) CELLS EXPOSED TO BROMACIL FOR 2 HOURS WITH METABOLIC ACTIVATION

Treatment	Cyto-geneticist[*]	No. of SCEs	No. of Chromosomes	Chromosomes/Cell (Mean ± SD)	SCEs/Cell[†] (Mean ± SE)	SCEs/ Chromosome[†] (Mean ± SE)
Negative control	A	423	491	19.64 ± 0.57	16.92 ± 0.82	0.862 ± 0.042
(.95% Ethanol)	B	392	493	19.72 ± 0.84	15.68 ± 0.79	0.795 ± 0.040
Bromacil						
78.125 µg/ml	A	447	488	19.52 ± 0.77	17.88 ± 0.85	0.916 ± 0.043
	B	385	487	19.48 ± 0.96	15.40 ± 0.78	0.791 ± 0.040
156.25 µg/ml	A	320	494	19.76 ± 0.83	12.80 ± 0.72	0.648 ± 0.036
	B	333	485	19.40 ± 1.08	13.32 ± 0.73	0.687 ± 0.038
312.5 µg/ml	A	436	493	19.72 ± 0.79	17.44 ± 0.84	0.884 ± 0.042
	B	364	492	19.68 ± 0.75	14.56 ± 0.76	0.740 ± 0.039
625 µg/ml	A	415	487	19.48 ± 1.05	16.60 ± 0.81	0.852 ± 0.042
	B	426	491	19.64 ± 1.44	17.04 ± 0.83	0.868 ± 0.042
1250 µg/ml	‡	--	--	--	--	--
	‡	--	--	--	--	--
Positive control						
(10^{-3} M dimethylnitrosamine)	A	814	489	19.56 ± 1.00	32.56 ± 1.14	1.665 ± 0.058
	B	758	498	19.92 ± 0.70	30.32 ± 1.10	1.522 ± 0.055

---

[*] Each cytogeneticist analyzed 25 cells per sample.

[†] A one-way analysis of variance comparing the SCE frequencies in CHO cells exposed to Bromacil and to the negative control indicated that the variance between treatment groups was not significantly greater than the variance within treatment groups.

[‡] Insufficient M_2 metaphase cells for analysis.

Table 68

MOUSE LYMPHOMA FORWARD MUTATION CONCENTRATION-RANGE ASSAY OF BROMACIL IN THE ABSENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency (× 10^{-6})	Induced Mutation Frequency (× 10^{-6})
Solvent control (1% DMSO)	100.0	600	603	83	100.5	100.5	28	--
	100.0	600	597	90	99.5	99.5	30	--
Positive control (500 μg/ml EMS)	72.2	600	428	838	71.3	51.5	392	363
	77.9	600	409	781	68.2	53.1	382	353
Bromacil								
1	104.7	600	579	136	96.5	101.0	47	18
2	109.3	600	594	157	99.0	108.2	53	24
5	110.0	600	612	106	102.0	112.2	35	6
10	109.3	600	671	155	111.8	122.2	46	17
20	110.0	600	549	107	91.5	100.7	39	10
40	115.8	600	577	133	96.2	111.4	46	17
80	104.4	600	537	152	89.5	93.4	57	28
100	110.0	600	608	156	101.3	111.4	51	22
150	109.3	600	590	149	98.3	107.5	51	22
200	104.4	600	556	165	92.7	96.7	59	30
300	104.4	600	634	122	105.7	110.3	38	9
400	99.8	600	579	107	96.5	96.3	37	8
500	58.8	600	638	96	106.3	62.5	30	1
600	82.0	600	592	143	98.7	80.9	48	19
700	68.0	600	558	123	93.0	63.2	44	15
800	46.2	600	545	173	90.8	42.0	63	34
900	10.3	600	469	268	78.2	8.1	114	85
1000	1.7	NC*	--	--	--	--	--	--

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*Not cloned.

Table 69

MOUSE LYMPHOMA FORWARD MUTATION CONCENTRATION-RANGE ASSAY OF BROMACIL IN THE PRESENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency (× 10⁻⁶)	Induced Mutation Frequency (× 10⁻⁶)
Solvent control (1% DMSO)	102.5	600	567	295	100.0	102.5	104	--
	97.5	600	567	324	100.0	97.5	114	--
Positive control (5 μg/ml 3-MCA)	54.9	600	361	644	63.7	35.0	357	248
	55.7	600	346	596	61.0	34.0	345	236
Bromacil								
1	106.1	600	517	214	91.2	96.7	83	---
2	108.0	600	546	292	96.3	104.0	107	---
5	104.5	600	598	343	105.5	110.2	115	6
10	93.0	600	554	336	97.7	90.9	121	12
20	88.6	600	508	424	89.6	79.4	167	58
40	31.9	600	281	485	49.6	15.8	345	236
80	1.5	NC*	--	--	--	--	--	--
100	1.7	NC	--	--	--	--	--	--
150	0.7	NC	--	--	--	--	--	--
200	0.8	NC	--	--	--	--	--	--
300	1.2	NC	--	--	--	--	--	--
400	1.6	NC	--	--	--	--	--	--
500	2.5	NC	--	--	--	--	--	--
600	5.3	NC	--	--	--	--	--	--
700	5.2	NC	--	--	--	--	--	--
800	5.1	NC	--	--	--	--	--	--
900	4.2	NC	--	--	--	--	--	--
1000	1.6	NC	--	--	--	--	--	--

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*Not cloned.

Table 70

MOUSE LYMPHOMA FORWARD MUTATION ASSAY OF BROMACIL IN THE ABSENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency ($\times 10^{-6}$)	Induced Mutation Frequency ($\times 10^{-6}$)
Solvent control (1% DMSO)	99.8	600	614	167	100.4	100.2	54	--
	100.2	600	609	176	99.6	99.8	58	--
Positive control (500 μg/ml EMS)	64.4	600	412	1207	67.4	43.4	586	530
	66.9	600	432	1353	70.6	47.3	626	570
Bromacil								
150	100.2	600	674	175	110.2	110.4	52	--
	95.7	600	617	189	100.9	96.6	61	5
400	84.2	600	603	233	98.6	83.0	77	21
	84.2	600	587	164	96.0	80.8	56	--
750	51.7	600	618	518	101.1	52.2	168	112
	54.6	600	601	492	98.3	53.7	164	108
850	36.7	600	542	--*	88.6	32.5	--	--
	23.8	600	601	746	98.3	23.4	248	192
880	11.1	600	493	960	80.6	8.9	389	333
	13.3	600	480	831	78.5	10.4	346	290
900	7.3	600	541	812	88.5	6.5	300	244
	8.8	600	522	886	85.4	7.5	339	283
930	3.9	600	489	644	80.0	3.1	263	207
	6.6	600	495	819	80.9	5.3	331	275
950	3.2	600	505	969	82.6	3.6	384	328
	4.4	NC†	--	--	--	--	--	--
980	1.2	NC	--	--	--	--	--	--
	1.5	NC	--	--	--	--	--	--
1000	1.1	NC	--	--	--	--	--	--
	1.0	NC	--	--	--	--	--	--

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*Sample lost
†Not cloned

Table 71

MOUSE LYMPHOMA FORWARD MUTATION ASSAY OF BROMACIL IN THE PRESENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency (× 10^{-6})	Induced Mutation Frequency (× 10^{-6})
Solvent control (1% DMSO)	101.4	600	560	234	101.2	102.6	84	--
	98.6	600	547	255	98.8	97.4	93	--
Positive control (6.0 μg/ml 3-MCA)	80.1	600	545	688	98.5	78.9	252	163
	79.1	600	475	730	85.8	67.9	307	218
Bromacil								
5	140.1	NC*	--	--	--	--	--	--
	133.6	NC	--	--	--	--	--	--
15	108.8	NC	--	--	--	--	--	--
	145.0	NC	--	--	--	--	--	--
20	90.2	600	568	353	102.6	92.6	124	35
	119.1	600	567	335	102.4	122.0	118	29
25	96.3	600	581	424	105.0	101.1	146	57
	119.1	600	636	405	114.9	136.4	127	38
35	99.1	600	670	530	121.0	120.0	158	69
	88.8	600	525	493	94.9	84.2	188	99
45	92.0	600	572	625	103.3	95.1	219	130
	86.0	600	574	612	103.7	89.2	213	124
55	65.5	600	492	730	88.9	58.2	297	208
	58.8	600	501	689	90.5	53.2	275	186
70	32.0	600	403	741	72.8	23.3	368	279
	39.5	600	405	750	73.2	28.9	370	281
80	30.7	600	326	736	58.9	18.1	452	363
	27.4	600	313	764	56.5	15.5	488	399
90	22.4	600	243	724	43.9	9.8	596	507
	25.0	600	250	677	45.2	11.3	542	453

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*Not cloned.

Table 72

DOSAGE AND SACRIFICE SCHEDULE

Compound	No. Animals	Dose at 0 and 24 Hr	Mean Body Weight Change (g)	No. Sacrificed 30 Hr	No. Sacrificed 48 Hr	No. Sacrificed 72 Hr	No. Fatalities
TMP--Positive control	12	1.25 g/kg	-1.6 ± 0.9	4	4	4	0
DMSO--Vehicle control	24	5 ml/kg	-0.7 ± 0.9	8	8	8	0
Bromacil							
Low Dose	28	75 mg/kg	-1.0 ± 0.5	8	8	8	0
Medium Dose	28	150 mg/kg	-1.3 ± 0.7	8	7	8	1
High Dose	28	300 mg/kg	-2.2 ± 0.5	8	6	4	10

Table 73

INCIDENCE OF MICRONUCLEATED POLYCHROMATOPHILIC ERYTHROCYTES
IN BLOOD AND BONE MARROW*
(500 PCEs Scored per Smear)

	30 Hours				48 Hour				72 Hours			
Dose	Animal No.	Blood PCE with MN	BM PCE with MN	BM PCE/RBC	Animal No.	Blood PCE with MN	BM PCE with MN	BM PCE/RBC	Animal No.	Blood PCE with MN	BM PCE with MN	BM PCE/RBC
DMSO 5 ml/kg	C0	+	0	1.3	A0	1	0	0.7	B0	1	1	0.9
	C1	+	0	1.0	A1	0	0	1.3	B1	1	0	1.2
	C2	+	1	1.0	A2	0	1	0.9	B2	0	1	0.6
	C3	+	1	0.7	A3	0	0	1.4	B3	0	1	0.9
	D0	+	2	1.0	E0	0	0	0.9	F0	0	1	2.7
	D1	+	0	1.3	E1	1	0	1.0	F1	0	1	1.2
	D2	+	1	1.0	E2	0	0	0.9	F2	0	0	2.8
	D3	+	3	1.2	E3	1	0	0.7	F3	0	2	0.9
	Total	--	8			3	1			2	8	
	Mean			1.1±.1				1.0±.3				1.4±.9
TMP 1.25 g/kg	J0	2	30	0.6	H0	4	21	0.7	I0	6	8	0.5
	J1	3	10	0.3	H1	0	13	0.6	I1	2	0	0.8
	J2	6	8	0.6	H2	0	20	1.3	I2	3	3	0.3
	J3	+	20	0.4	H3	0	3	0.2	I3	1	0	0.7
	Total	11	68			4	57			12	11	
	Mean			0.5±.15				0.7±.5				0.6±.2
Bromacil 75 mg/kg	K0	+	0	1.4	N0	0	0	1.3	L0	0	0	1.8
	K1	+	1	1.1	N1	0	1	2.2	L1	0	0	1.4
	K2	+	0	0.8	N2	0	0	0.8	L2	0	1	1.4
	K3	+	0	0.9	N3	2	0	1.2	L3	0	0	1.4
	M0	0	0	0.5	Q0	0	1	0.5	O0	1	1	1.0
	M1	1	1	0.6	Q1	1	0	0.7	O1	2	0	0.5
	M2	0	1	0.9	Q2	0	1	0.9	O2	0	0	1.1
	M3	0	0	1.2	Q3	0	3	0.9	O3	0	0	1.0
	Total	1	3			3	6			3	2	
	Mean			0.9±.3				1.1±.5				1.2±.4
Bromacil 150 mg/kg	T0	+	0	1.1	S0	0	0	1.8	R0	0	1	0.4
	T1	+	2	0.9	S1	1	0	0.3	R1	0	0	1.2
	T2	1	0	1.0	S2	2	1	0.4	R2	1	0	0.8
	T3	0	1	0.9	V0	0	1	0.8	R3	0	0	0.9
	X0	0	0	0.7	V1	0	2	0.8	W0	0	0	0.7
	X1	0	1	0.7	V2	1	0	1.3	W1	0	0	1.3
	X2	1	1	1.4	V3	0	0	1.0	W2	+	2	0.8
	X3	1	0	1.0					W3	0	0	0.7
	Total	3	5			4	4			1	3	
	Mean			1.0±.2				0.9±.5				0.9±0.3
Bromacil 300 mg/kg	Z0	2	0	1.1	Y0	0	0	1.1	BB0‡	2	1	0.9
	Z1	0	2	1.1	Y1	1	0	0.9	CC0	2	1.5	0.2
	Z2	1	1	0.7	AA2	0	1	0.5	CC1	0	1.5	0.6
	Z3	2	1	0.3	AA3	0	1	0.9	CC3	1	1	0.3
	EE0	1	1	0.6	BB1	0	1	2.1				
	EE1	1	0	0.6	DD1	0	0	0.8				
	EE2	1	1	0.5								
	EE3	2	1	0.7								
	Total	10	7			1	3			5	5	
	Mean			0.7±.3				1.1±.6				0.5±.3

---

*BM = bone marrow; PCE = polychromatophilic erythrocyte; RBC = mature erythrocyte; MN = micronucleus.
+Blood smears were not prepared for these animals.
‡Bone marrow values for this group are based on 1000 cells scored per smear.

sec-Butylamine (2-AB)

S. typhimurium Plate Incorporation, E. coli WP-2 (Tables 74, 75). The compound was tested once over a range of 0.1 to 50.0 µℓ/plate (0.07 to 36.5 mg/plate, density = 0.73) and retested at doses ranging from 0.1 to 25.0 µℓ/plate (~ 0.07 to 18.3 mg/plate). No reproducible, dose-related increase in the number of revertants was observed in either of these tests. Toxicity was seen with all strains at 25.0 µℓ/plate in the plate incorporation assay. In the WP-2 system, toxicity was seen at 50.0 µℓ/plate.

S. cerevisiae D3 (Tables 76, 77). No recombinogenic activity was observed with this compound when it was tested over concentrations ranging from 0.05 to 0.5% and retested from 0.05 to 0.18%. Toxicity was observed at 0.2% without metabolic activation and at 0.3% with metabolic activation.

E. coli/B. subtilis Relative Toxicity (Tables 78-80). This compound was tested at concentrations of 10 and 20 µℓ, then at 25 and 50 µℓ. It was more toxic to the repair-deficient than to the repair-proficient strain in E. coli. This positive response was both reproducible and concentration-related. No differential toxicity was observed in the B. subtilis strains.

Table 74

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND E. COLI

sec-BUTYLAMINE(2-AB)

Experiment 1

Compound	Metabolic Activation	Amount of Compound Added per Plate	Salmonella typhimurium Histidine Revertants per Plate TA1535		TA1537		TA1538		TA98		TA100		E. coli Tryptophan Revertants per Plate WP2	
Negative Control														
H₂O	-	50.0 µl	15	15	2	10	13	15	33	26	127	116	28	34
	+	50.0	18	15	17	16	20	12	36	41	102	96	50	41
Positive Controls														
Sodium Azide	-	0.5 µg	125	123										
	-	1.0									386	366		
9-Aminoacridine	-	50.0			194	88								
2-Nitrofluorene	-	5.0					737	780	370	437				
AF-2	-	0.1											1375	1434
2-Anthramine	-	1.0					25	20	28	42	110	111		
	+	1.0					338	430	246	287	390	420		
	-	2.5	20	17	9	9								
	+	2.5	160	153	127	131								
	-	10.0											36	40
	+	10.0											375	370
sec-Butylamine(2-AB)	-	0.1 µl	28	18	5	10	15	14	25	36	116	112	33	41
	-	0.5	14	12	10	8	28	10	31	28	103	92	34	37
	-	1.0	13	17	11	8	15	10	26	33	105	96	34	32
	-	5.0	21	15	8	14	12	14	17	30	100	89	36	48
	-	10.0	15	6	5	9	14	10	17	20	125	111	36	31
	-	50.0	PP*	PP	PP	PP	PP	PP	PP	PP	PP	PP	PP	PP
	+	0.1 µl	17	8	12	14	26	20	51	42	116	112	49	48
	+	0.5	13	15	18	14	21	32	49	51	109	114	39	46
	+	1.0	21	9	19	16	17	18	50	37	110	113	55	44
	+	5.0	10	15	14	14	14	24	40	40	101	135	52	52
	+	10.0	19	18	16	15	21	33	40	37	124	140	45	58
	+	50.0	PP	PP	PP	PP	PP	PP	PP	PP	PP	PP	PP	PP

---

*PP = pinpoint colonies on background.

Table 75

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND E. COLI

sec-BUTYLAMINE(2-AB)

Experiment 2

Compound	Metabolic Activation	Amount of Compound Added per Plate	Salmonella typhimurium Histidine Revertants per Plate										E. coli Tryptophan Revertants per Plate	
			TA1535		TA1537		TA1538		TA98		TA100		WP2	
Negative Control														
H_2O	-	50.0 µl	28	16	24	14	12	13	44	30	126	116	27	27
	+	50.0	12	20	40	19	26	31	53	45	125	128	55	60
Positive Controls														
Sodium Azide	-	0.5 µg	233	247										
	-	1.0									347	349		
9-Aminoacridine	-	50.0			135	150								
2-Nitrofluorene	-	5.0					768	821	446	427				
AF-2	-	0.1											1294	1363
2-Anthramine	-	1.0					18	17	20	26	131	142		
	+	1.0					187	238	245	260	592	494		
	-	2.5	26	26	33	18								
	+	2.5	195	207	149	125								
	-	10.0											31	32
	+	10.0											596	542
sec-Butylamine(2-AB)	-	0.1 µl	26	30	9	7	8	12	31	24	105	92	35	29
	-	0.5	25	29	7	3	9	10	17	20	90	98	37	42
	-	1.0	24	20	8	5	10	7	38	27	80	91	32	29
	-	5.0	16	19	9	6	5	6	24	14	81	89	29	30
	-	10.0	25	18	10	8	10	10	12	28	93	79	36	31
	-	25.0	PP*	PP	PP	PP	PP	PP	PP	PP	PP	PP	PP	PP
	+	0.1 µl	14	18	18	28	13	19	40	48	104	125	51	39
	+	0.5	14	14	12	15	27	27	36	31	109	110	43	64
	+	1.0	9	13	7	15	26	26	27	40	108	102	45	48
	+	5.0	16	20	16	16	25	21	31	29	85	111	51	54
	+	10.0	28	28	7	19	24	17	32	12	91	112	42	57
	+	25.0	PP	PP	PP	PP	PP	PP	PP	PP	PP	PP	44	52

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*PP = pinpoint colonies on background.

Table 76

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

sec-BUTYLAMINE(2-AB)
Experiment 1

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml (x 10^{-7})	Percent	Per ml (x 10^{-3})	Per 10^5 Survivors
Negative Control						
H_2O	-		7.6	100	1.5	2.0
	+		7.7	100	4.0	5.2
Positive Controls						
1,2,3,4-Diepoxybutane	-	0.025	7.8	103	650	830
	+	0.025	8.9	117	280	310
sec-Butylamine(2-AB)	-	0.05	8.8	116	2.0	2.5
	-	0.10	7.9	104	2.0	2.5
	-	0.20	T*	T	T	T
	-	0.30	T	T	T	T
	-	0.40	T	T	T	T
	-	0.50	T	T	T	T
	+	0.05	6.3	82	1.0	1.6
	+	0.10	8.3	104	5.0	6.3
	+	0.20	5.8	75	3.0	5.2
	+	0.30	T	T	T	T
	+	0.40	T	T	T	T
	+	0.50	T	T	T	T

---

*T = toxic.

Table 77

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

sec-BUTYLAMINE(2-AB)
Experiment 2

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($x\ 10^{-7}$)	Percent	Per ml ($x\ 10^{-3}$)	Per 10^5 Survivors
Negative Control						
H_2O	-		7.6	100	6.0	7.9
	+		7.0	100	3.5	5.0
Positive Controls						
1,2,3,4-Diepoxybutane	-	0.025	6.9	79	8.1	14
	+	0.025	6.6	94	7.9	13
sec-Butylamine(2-AB)	-	0.05	7.4	97	3.0	4.1
	-	0.10	8.5	112	5.0	5.9
	-	0.12	8.2	108	4.0	4.9
	-	0.14	7.2	95	3.0	4.2
	-	0.16	7.2	95	3.0	4.2
	-	0.18	5.9	78	4.0	6.8
	+	0.05	5.5	79	6.0	1.1
	+	0.10	8.0	114	5.0	6.3
	+	0.12	7.1	101	3.0	4.2
	+	0.14	7.5	107	1.0	1.3
	+	0.16	5.5	79	4.0	7.3
	+	0.18	5.8	83	3.0	5.2

TABLE 78

DIFFERENTIAL TOXICITY ASSAY

Sec-BUTYLAMINE(2-AB)

Experiment 1

Compound	Amount of Compound Added per Plate	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		E. coli				B. subtilis			
		W3110 (polA$^+$)		p3478 (polA$^-$)		H17 (rec$^+$)		M45 (rec$^-$)	
Negative Controls									
Ampicillin	20 μg	21	20	20	21				
Chloramphenicol	20 μg					31	31	29	30
Positive Control									
Methylmethanesulfonate	1 mg	33	30	38	36	16	15	20	20
sec-Butylamine(2-AB)	10 μℓ	14	12	14	17	12	11	11	10
	20	21	16	24	24	18	17	16	16

TABLE 79

DIFFERENTIAL TOXICITY ASSAY

Sec-BUTYLAMINE(2-AB)

Experiment 2

Compound	Amount of Compound Added per Plate	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		E. coli				B. subtilis			
		W3110 (polA$^+$)		p3478 (polA$^-$)		H17 (rec$^+$)		M45 (rec$^-$)	
Negative Control									
Chloramphenicol	20 µg	25	25	25	24	30	30	30	29
Positive Control									
Methylmethanesulfonate	1 mg	13	15	32	26	13	13	20	17
sec-Butylamine(2-AB)	10 µℓ	17	18	20	22	13	14	13	14
	20	25	25	28	28	17	17	18	19

TABLE 80

DIFFERENTIAL TOXICITY ASSAY

Sec-BUTYLAMINE(2-AB)

Experiment 3

Compound	Amount of Compound Added per Plate	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		E. coli				B. subtilis			
		W3110 (polA$^+$)		p3478 (polA$^-$)		H17 (rec$^+$)		M45 (rec$^-$)	
Negative Control									
Ampicillin	20 μg	20	20	18	18	30	31	29	30
Positive Control									
Methylmethanesulfonate	1 mg	31	31	35	34	14	16	21	20
sec-Butylamine(2-AB)	25 μℓ	24	23	32	31	16	15	16	16
	50	33	32	42	41	27	25	25	25

sec-Butylamine (2-AB)$^{+H_3PO_4}$

S. typhimurium Plate Incorporation, E. coli WP-2 (Tables 81, 82). The compound was first tested over a concentration range of 1 to 100 μℓ/plate. A second experiment was conducted over the same range for all strains except TA98 and TA100, which were tested up to 500 μℓ/plate. This was due to the shortage of sample remaining and the desirability of testing for toxicity with strains TA98 and TA100. A toxic response was obtained at a concentration of 500 μℓ/plate with these two strains, both with and without metabolic activation, but no increase in the number of revertants was observed in either test.

S. cerevisiae D3 (Tables 83, 84). The compound was first tested at concentrations ranging from 0.1 to 5.0% and then retested at concentrations ranging from 1.0 to 7.5%. No reproducible dose-related increase in the number of mitotic recombinants was observed with sec-butylamine(2-AB)$^{+H_3PO_4}$.

E. coli/B. subtilis Relative Toxicity (Tables 85-87). A negative response was obtained for this compound in both species in the relative toxicity assay.

Table 81

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND E. COLI

sec-BUTYLAMINE(2-AB)$^{+H_3PO_4}$

Experiment 1

Compound	Metabolic Activation	Amount of Compound Added per Plate	Salmonella typhimurium Histidine Revertants per Plate TA1535		TA1537		TA1538		TA98		TA100		E. coli Tryptophan Revertants per Plate WP2	
Negative Control														
H_2O	-	100.0 µl	20	17	21	16	21	22	43	50	115	105	55	58
	+	100.0	12	11	6	13	19	18	26	21	102	100	37	31
Positive Controls														
Sodium Azide	-	0.5 µg	125	123										
	-	1.0									386	366		
9-Aminoacridine	-	50.0			194	88								
2-Nitrofluorene	-	5.0					737	780	370	437				
AF-2	-	0.1											1375	1434
2-Anthramine	-	1.0					25	20	28	42	110	111		
	+	1.0					338	430	246	287	390	420		
	-	2.5	20	17	9	9								
	+	2.5	160	153	127	131								
	-	10.0											36	40
	+	10.0											375	370
sec-Butylamine(2-AB)$^{+H_3PO_4}$	-	1.0 µl	21	13	9	8	14	18	28	25	137	136	29	34
	-	5.0	17	24	8	8	12	12	31	29	138	122	30	32
	-	10.0	19	27	6	18	9	8	25	31	100	124	29	24
	-	25.0	18	21	19	8	9	13	24	32	104	125	29	26
	-	50.0	24	15	9	8	7	10	25	26	116	108	32	33
	-	100.0	26	21	8	16	8	20	14	31	108	109	28	27
	+	1.0 µl	13	17	13	17	17	17	31	38	136	115	34	43
	+	5.0	13	14	9	21	16	21	40	31	136	115	40	43
	+	10.0	16	16	18	28	30	28	43	39	124	111	49	39
	+	25.0	9	13	18	25	15	26	50	51	117	116	48	53
	+	50.0	14	12	15	16	16	21	43	37	125	100	52	55
	+	100.0	14	14	18	21	21	19	40	37	90	115	50	41

Table 82

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND E. COLI

sec-BUTYLAMINE(2-AB)$^{+H_3PO_4}$

Experiment 2

Compound	Metabolic Activation	Amount of Compound Added per Plate	Salmonella typhimurium Histidine Revertants per Plate										E. coli Tryptophan Revertants per Plate	
			TA1535		TA1537		TA1538		TA98		TA100		WP2	
Negative Control														
H_2O	-	500.0 µl	32	28	13	21	13	18	32	29	134	113	21	28
	+	500.0	19	16	28	26	25	18	42	52	149	152	54	49
Positive Controls														
Sodium Azide	-	0.5 µg	233	247										
	-	1.0									347	349		
9-Aminoacridine	-	50.0			135	150								
2-Nitrofluorene	-	5.0					768	821	446	427				
AF-2	-	0.1											1294	1363
2-Anthramine	-	1.0					18	17	20	26	131	142		
	+	1.0					187	238	245	260	592	493		
	-	2.5	26	26	33	18								
	+	2.5	195	207	149	125								
	-	10.0											31	32
	+	10.0											596	542
sec-Butylamine(2-AB)$^{+H_3PO_4}$	-	1.0 µl	20	32	12	15	7	16	28	31	90	110	30	33
	-	5.0	14	21	8	10	13	11	29	26	110	89	38	38
	-	10.0	21	24	12	10	11	18	21	24	103	112	39	43
	-	25.0	21	33	13	12	9	14	30	30	91	101	38	45
	-	50.0	19	24	8	10	14	16	25	29	92	98	38	41
	-	100.0	25	13	14	8	15	9	20	27	48	79	26	40
	-	500.0	NT[*]	NT	NT	NT	NT	NT	PP[†]	PP	PP	PP	NT	NT
	+	1.0 µl	18	15	26	21	26	25	48	43	115	130	51	54
	+	5.0	10	12	21	25	30	32	39	45	123	127	57	59
	+	10.0	10	13	25	27	30	28	32	50	110	123	54	63
	+	25.0	12	17	27	16	20	18	43	41	108	127	65	52
	+	50.0	16	12	21	21	19	24	43	38	101	101	56	65
	+	100.0	13	14	16	15	29	24	38	38	80	112	55	49
	+	500.0	NT	NT	NT	NT	NT	NT	PP	PP	PP	PP	NT	NT

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[*] NT = not tested.

[†] PP = pinpoint colonies.

Table 83

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

sec-BUTYLAMINE(2-AB)$^{+H_3PO_4}$
Experiment 1

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml (x 10^{-7})	Percent	Per ml (x 10^{-3})	Per 10^5 Survivors
Negative Control						
H_2O	-		8.5	100	6.5	7.7
	+		8.5	100	4.5	5.3
Positive Controls						
1,2,3,4-Diepoxybutane	-	0.025	7.8	92	600	770
	+	0.025	9.4	111	600	640
sec-Butylamine(2-AB)$^{+H_3PO_4}$	-	0.1	11.0	129	4.0	3.6
	-	0.5	9.9	116	3.0	3.0
	-	1.0	9.5	112	6.0	6.3
	-	5.0	6.4	75	7.0	11
	+	0.1	7.1	84	5.0	7.0
	+	0.5	9.6	113	6.0	6.3
	+	1.0	9.3	109	4.0	4.3
	+	5.0	6.5	76	4.0	6.2

Table 84

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

sec-BUTYLAMINE(2-AB)$^{+H_3PO_4}$
Experiment 2

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative Control						
H$_2$O	-		7.6	100	1.5	2.0
	+		7.7	100	4.0	5.2
Positive Controls						
1,2,3,4-Diepoxybutane	-	0.025	7.8	103	650	830
	+	0.025	8.9	117	280	310
sec-Butylamine(2-AB)$^{+H_3PO_4}$	-	1.0	6.7	88	2.0	3.0
	-	2.5	5.9	78	5.0	8.5
	-	5.0	7.3	96	7.0	9.6
	-	7.5	5.6	74	1.0	1.8
	+	1.0	5.2	68	4.0	7.7
	+	2.5	7.5	97	4.0	5.3
	+	5.0	6.2	81	3.0	4.8
	+	7.5	5.7	74	7.0	12

TABLE 85

DIFFERENTIAL TOXICITY ASSAY

Sec-BUTYLAMINE(2-AB)$^{+H_3PO_4}$

Experiment 1

Compound	Amount of Compound Added per Plate	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		E. coli				B. subtilis			
		W3110 (polA$^+$)		p3478 (polA$^-$)		H17 (rec$^+$)		M45 (rec$^-$)	
Negative Controls									
Ampicillin	20 µg	21	20	20	21				
Chloramphenicol	20 µg					31	31	29	30
Positive Control									
Methylmethanesulfonate	1 mg	33	30	38	36	16	15	20	20
sec-Butylamine(2-AB)$^{+H_3PO_4}$	10 µℓ	7	7	7	8	8	9	9	10
	20	7	8	9	9	10	10	10	10

TABLE 86

DIFFERENTIAL TOXICITY ASSAY

Sec-BUTYLAMINE(2-AB)$^{+H_3PO_4}$

Experiment 2

Compound	Amount of Compound Added per Plate	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		E. coli				B. subtilis			
		W3110 (polA$^+$)		p3478 (polA$^-$)		H17 (rec$^+$)		M45 (rec$^-$)	
Negative Control									
Chloramphenicol	20 µg	25	25	25	24	30	30	30	29
Positive Control									
Methylmethanesulfonate	1 mg	13	15	32	26	13	13	20	17
sec-Butylamine(2-AB)$^{+H_3PO_4}$	10 µℓ	7	7	7	7	7	7	7	8
	20	8	8	9	9	9	9	9	10

TABLE 87

DIFFERENTIAL TOXICITY ASSAY

Sec-BUTYLAMINE(2-AB)$^{+H_3PO_4}$

Experiment 3

Compound	Amount of Compound Added per Plate	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		E. coli				B. subtilis			
		W3110 (polA$^+$)		p3478 (polA$^-$)		H17 (rec$^+$)		M45 (rec$^-$)	
Negative Control									
Ampicillin	20 µg	20	20	18	18	30	31	29	30
Positive Control									
Methylmethanesulfonate	1 mg	31	31	35	34	14	16	21	20
sec-Butylamine(2-AB)$^{+H_3PO_4}$	25 µℓ	7	7	7	7	7	7	8	7
	50	8	9	9	9	8	9	9	9

Cacodylic Acid

S. typhimurium Plate Incorporation (Table 88). This compound had been previously tested in strains TA100, TA1535, TA1537 and TA1538 and found negative in all strains, both with and without activation (51). Under the present contract it was retested in strains TA98 and TA100. It was negative in both strains with and without activation.

E. coli WP-2. Cacodylic acid was previously tested and found to be negative, both with and without activation (51).

S. cerevisiae D3 (Table 89). This compound was previously tested in this system over a narrow dose range and found to be positive, both with and without activation (51). It was retested under the present contract over a wider dose range, with the same conclusion.

S. cerevisiae D7 (Tables 90, 91). A concentration range of 0.5 to 5.0% was chosen for the first experiment and 0.5 to 2.0% for the second. This compound elicited a positive response with and without activation in all three genetic end points in the D7 strain.

E. coli/B. subtilis Relative Toxicity. Cacodylic acid was previously tested in this system and was reported to be negative in both species (51). Reexamination of the data indicates that this test should be more appropriately regarded as inconclusive.

S. typhimurium Relative Toxicity (Tables 92, 93). This compound was found to be negative in both strain pairs in this assay.

SCE in CHO Cells (Tables 94-98). Cacodylic acid was dissolved in 95% ethanol at a concentration of 368 mg/ml on the day of the cytotoxicity test without metabolic activation. However, on the day of the cytotoxicity test with metabolic activation, the maximum concentration of cacodylic acid dissolved in 95% ethanol achieved was 307.35 mg/ml. This discrepancy may have been due to the lower room temperature on the day of the latter experiment. At a concentration of $\leq$ 3680 µg/ml, cacodylic acid did not precipitate in the culture medium or in the metabolic activation mixture.

Table 94 presents the results of the cytotoxicity test of cacodylic acid in CHO cell cultures without metabolic activation. At concentrations of 3680 µg/ml, cacodylic acid was cytotoxic to CHO cells; at a concentration of 736 µg/ml, the culture yielded primarily M_1 metaphases. At 147.2 µg/ml or less, M_2 metaphase cells were produced. Table 95 presents the results of the cytotoxicity test of cacodylic acid in CHO cell cultures with metabolic activation. The highest concentration of cacodylic acid tested, 3073.5 µg/ml, was not toxic to CHO cells and produced primarily M_2 metaphase cells, as did the four lower concentrations.

Table 96 presents the SCE frequencies in CHO cells exposed to cacodylic acid without metabolic activation. A one-way analysis of variance was conducted on SCE frequencies of the five concentrations of cacodylic acid and of the negative controls. The variance between treatment groups was not significantly greater than the variance within treatment groups. Because there was a discrepancy between the trends observed by the two cytogeneticists

(B's scores indicated an apparent elevation in SCE frequencies with increasing concentration, while A's scores indicated an opposite trend), a third cytogeneticist was asked to evaluate the coded slides. Table 97 presents the observations of all three cytogeneticists. C's scores did not indicate any consistent change in SCE frequency with concentration. Analysis of the combined results for all three cytogeneticists revealed that the variance between treatment groups was not greater than the variance within treatment groups.

Table 98 presents the SCE frequencies in CHO cells exposed to cacodylic acid with metabolic activation. At a concentration of 3000 μg/ml, the number of M_2 metaphase cells produced was not sufficient for analysis. A one-way analysis of variance was conducted on SCE frequencies of the four lower concentrations of cacodylic acid and of the negative controls. The variance between treatment groups was not significantly greater than the variance within treatment groups. No trend in SCE frequency with concentration was apparent.

We conclude that cacodylic acid did not induce SCEs in CHO cells, either with or without metabolic activation and therefore is negative in this test system.

L5178Y Mouse Lymphoma (Tables 99-102). The samples for the concentration-range tests of cacodylic acid in the absence of the S-9 metabolic activation system were not cloned, due to a technical error. The cytotoxicity results showed that the range to use in the mutgenesis assay was approximately 150 to 1000 μg/ml (99.5% relative suspension growth at 150 μg/ml and 16.0% relative suspension growth at 1000 μg/ml). Concentration-range test results for cacodylic acid in the presence of metabolic activation system (Table 99) showed that the approximate range to use in the mutagenesis assay was from less than 500 μg/ml to over 1000 μg/ml (93.7% relative total growth at 500 μg/ml and 34.7% relative total growth at 1000 μg/ml).

Tables 100 and 101 present the results of mutagenesis tests of cacodylic acid in the absence of the S-9 metabolic activation system. In the first assay, the highest concentration (1000 μg/ml) yielded an average relative total growth of 9.9%. In the repeat assay, this compound was extremely toxic at concentrations greater than 900 μg/ml, yielding relative total growth values of less than 5.0%. No significant depression in relative total growth was observed at 150 μg/ml in the first assay and at 105 μg/ml in the repeat assay. These concentrations yielded average relative total growth values of 84.9% and 92.9%, respectively. From approximately 105 μg/ml to 1000 μg/ml, a concentration-related increase in induced mutation frequency was observed in both assays. Average mutation frequencies of at least twice the spontaneous frequency were observed at 900 and 1000 μg/ml (the two highest concentrations) in the first assay and at 900 μg/ml in the repeat assay, with average relative total growth values of approximately 10.0% or greater.

Table 102 presents the results of mutagenesis tests in the presence of the S-9 metabolic activation system. The highest concentration (1400 μg/ml) yielded an average relative total growth value of 33.8%. No significant depression in relative total growth was observed at 420 μg/ml, which yielded an average relative total growth value of 91.7%. Between 420 and 1400 μg/ml, a concentration-related increase in induced mutation frequency was observed.

Average mutation frequencies at least twice the spontaneous frequency were observed from 1190 to 1400 μg/ml (the highest three concentrations).

These combined results indicate a mutagenic effect of cacodylic acid at the thymidine kinase locus in L5178Y mouse lymphoma cells, both with and without the metabolic activation system. In both cases, cacodylic acid increased the mutation frequency, in a concentration-related manner, above the spontaneous frequencies observed in the solvent controls. The presence of the metabolic activation system appeared to enhance the mutagenic response and decrease the cytotoxic response to cacodylic acid.

UDS in WI-38 Cells. Cacodylic acid was found to be negative both with and without activation in tests conducted previously (51).

Micronucleus Test (Tables 103-104). The dosing and sacrifice schedules are shown in Table 103. The incidences of micronuclei in PCEs and the PCE to RBC ratios are given in Table 104.

The animals gained weight and showed no signs of toxicity 24 hours after the first dose. Therefore, the second dose was doubled to ensure meeting the criteria for a maximum tolerated dose, should the compound be negative.

Cacodylic acid was positive in the high dose (750 + 1500 mg/kg) groups 48 and 72 hours after first administration. High mortality at this dose level precluded evaluating a 96-hour group. The low and medium doses of cacodylic acid were negative 48 hours, positive 72 hours, and equivocal 96 hours after first administration. The vehicle control groups were negative at all times ($\beta < 0.05$). The PCE to RBC ratios are within normal limits ($\bar{x} = 0.5$ to 1.5) at all times and dose levels. Because four dose-time groups were in the positive range ($\alpha < 0.05$), cacodylic acid meets the criteria for mutagenesis in the mouse micronucleus test.

Table 88

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

CACODYLIC ACID

Retest

Compound	Metabolic Activation	Compound Added (µg/plate)	Histidine Revertants per Plate TA1535	TA1537	TA1538	TA98	TA100
Negative Control							
DMSO	-					28 30	135 138
	+					28 38	139 132
Positive Controls:							
2-Anthramine	-	1.0				42	145
	+	1.0				324	746
Sodium Azide	-	0.5					397
2-Nitrofluorene	-	5.0				543	
Cacodylic Acid	-	500.0				27	110
		1,000.0				24	128
	-	2,000.0				25	130
	-	3,000.0				26	125
	-	4,000.0				28	142
	-	5,000.0				31	167
	+	500.0				39	117
	+	1,000.0				36	131
	+	2,000.0				35	115
	+	3,000.0				43	134
	+	4,000.0				31	120
	+	5,000.0				27	108

Table 89

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

CACODYLIC ACID

Retest

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml $(x\ 10^{-7})$	Percent	Per ml $(x\ 10^{-3})$	Per 10^5 Survivors
Negative Control: DMSO	-		7.7	100	3.5	4.6
	+		7.4	100	3.5	4.7
Positive Control 1,2,3,4-Diepoxybutane	-	0.025	7.0	85	415	592.9
	+		7.3	82	412.5	565.1
Cacodylic Acid	-	1	9.7	126	3.0	3.1
	-	2	7.3	95	586.0	802.7
	-	3	6.9	90	996.0	1443.5
	-	4	5.4	70	1295.0	2398.2
	+	1	7.4	100	11.0	14.9
	+	2	8.3	112	650.0	783.1
	+	3	8.5	115	1045.0	1229.4
	+	4	5.9	80	1493.3	2531.1

Table 90

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D7

CACODYLIC ACID

Experiment 1

			Survivors		Crossing-Over				Gene Conversion		Reverse Mutation	
					Mitotic Recombinants		Total Aberrants		Convertants		Revertants	
Compound	MA	% Conc.	Cells Per ml ($\times 10^{-7}$)	%	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors	Per ml ($\times 10^{-1}$)	Per 10^6 Survivors	Per ml ($\times 10^0$)	Per 10^7 Survivors
Negative Control												
H_2O	-	10.0	5.6	100	0.50	0.89	16	29	39	7.0	17	3.0
	+	10.0	5.6	100	0.50	0.89	14	25	31	5.5	17	3.0
Positive Control												
1,2,3,4-Diepoxybutane	-	0.013	4.6	82	97	210	810	1800	1700	370	350	76
	+	0.013	6.3	113	100	160	870	1400	1600	250	310	49
Cacodylic Acid	-	0.5	6.1	109	1.0	1.6	24	39	88	14	58	9.5
	-	1.0	4.7	84	150	320	370	790	1700	360	110	23
	-	2.0	3.4	61	150	440	450	1300	1900	560	110	32
	-	4.0	2.8	50	110	390	300	1100	1600	570	94	34
	-	5.0	1.5	27	83	550	220	1500	1200	800	80	53
	+	0.5	5.5	98	1.0	1.8	20	36	66	12	58	11
	+	1.0	5.7	102	24	42	89	160	520	91	93	16
	+	2.0	4.5	80	160	360	420	93	2000	440	95	21
	+	4.0	2.9	52	100	340	290	1000	1500	520	53	18
	+	5.0	2.6	46	90	350	330	1300	1600	620	92	35

Table 91

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D7

CACODYLIC ACID

Experiment 2

Compound	MA	% Conc.	Survivors		Crossing-Over				Gene Conversion		Reverse Mutation	
			Cells Per ml (× 10^{-7})	%	Mitotic Recombinants		Total Aberrants		Convertants		Revertants	
					Per ml (× 10^{-3})	Per 10^5 Survivors	Per ml (× 10^{-3})	Per 10^5 Survivors	Per ml (× 10^{-1})	Per 10^6 Survivors	Per ml (× 10^0)	Per 10^7 Survivors
Negative Control												
H_2O	-	10.0	5.6	100	0	0	12	21	39	7.0	9.0	1.6
	+	10.0	5.7	100	0	0	11	19	41	7.2	7.5	1.3
Positive Control												
1,2,3,4-Diepoxybutane	-	0.013	4.1	73	160	390	660	1600	1000	240	180	44
	+	0.013	5.2	91	110	210	550	1100	1500	290	150	29
Cacodylic Acid	-	0.50	6.9	123	9.0	13	42	61	350	51	66	9.6
	-	0.75	6.7	120	73	110	280	420	2000	300	130	19
	-	1.0	4.0	71	92	230	560	1400	2200	550	170	43
	-	1.5	3.8	68	97	260	410	1100	2200	580	130	34
	-	2.0	3.3	59	94	280	520	160	2100	640	160	48
	+	0.50	7.7	135	3.0	3.9	23	30	90	12	38	4.9
	+	0.75	6.9	121	35	51	230	330	200	29	54	7.8
	+	1.0	7.2	126	50	69	380	530	1100	150	110	15
	+	1.5	5.8	102	83	140	530	910	2700	470	120	20
	+	2.0	4.5	79	85	190	480	1100	2300	510	120	27

Table 92

DIFFERENTIAL TOXICITY ASSAYS WITH SALMONELLA TYPHIMURIUM STRAINS

Experiment 1

Compound	Amount of Compound Applied to Disc	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		SL4525 (rec$^+$)		SL4700 (rec$^-$)		TA1978 (uvrB$^+$)		TA1538 (uvrB$^-$)	
Negative Control									
Kanamycin	100.0 μg	14	13	13	14	13	13	14	14
Positive Controls									
MMS*	10.0 mg	47	46	68	65				
4-NQO†	2.0 mg					12	12	24	23
Cacodylic Acid	1.0 mg	13	10	10	12	11	10	11	10
	5.0 mg	25	28	30	28	24	23	23	25

Table 93

DIFFERENTIAL TOXICITY ASSAYS WITH SALMONELLA TYPHIMURIUM STRAINS

Experiment 2

Compound	Amount of Compound Applied to Disc	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		SL4525 (rec$^+$)		SL4700 (rec$^-$)		TA1978 (uvrB$^+$)		TA1538 (uvrB$^-$)	
Negative Control									
Kanamycin	100.0 µg	15	14	16	15	17	17	15	16
Positive Controls									
MMS*	10.0 mg	45	40	72	74				
4-NQO†	2.0 mg					12	12	28	26
Cacodylic Acid	1.0 mg	20	19	18	16	10	11	14	15
	5.0 mg	30	27	30	31	24	25	25	25

Table 94

CYTOTOXICITY TEST OF CHINESE HAMSTER OVARY CELLS EXPOSED TO CACODYLIC ACID FOR 21.5 HOURS WITHOUT METABOLIC ACTIVATION

Treatment	Number of Divisions in Bromodeoxyuridine (BrdU)[*]
Negative control (0.95% ethanol)	M_2
Cacodylic acid (μg/ml)	
5.9	M_2
29.4	$M_2 >> M_1$[†]
147.2	$M_2 >> M_1$[†]
736	$M_1 >> M_2$[†]
3680	--[‡]
Positive control (10^{-3} M ethyl methanesulfonate)	M_2

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[*] M_1, one division in BrdU; M_2, two divisions in BrdU.

[†] The cell type to the left of the >> sign was in great excess.

[‡] Flask was not harvested due to excessive cytotoxicity.

Table 95

CYTOTOXICITY TEST OF CHINESE HAMSTER OVARY CELLS EXPOSED TO CACODYLIC ACID FOR 2 HOURS WITH METABOLIC ACTIVATION

Treatment	Number of Divisions in Bromodeoxyuridine (BrdU)*
Negative control (0.95% ethanol)	M_2
Cacodylic acid (μg/ml)	
4.9	M_2
24.6	M_2
122.9	M_2
614.7	M_2
3073.5	M_2
Positive control (10^{-3} M dimethylnitrosamine)	M_2

---

*M_2, two divisions in BrdU.

Table 96

SISTER CHROMATID EXCHANGE (SCE) FREQUENCIES IN CHINESE HAMSTER OVARY (CHO) CELLS EXPOSED TO CACODYLIC ACID FOR 21.5 HOURS WITHOUT METABOLIC ACTIVATION

Treatment	Cytogeneticist*	No. of SCEs	No. of Chromosomes	Chromosomes/Cell (Mean ± SD)	SCEs/Cell† (Mean ± SE)	SCEs/Chromosome† (Mean ± SE)
Negative control (0.95% ethanol)	A	282	476	19.04 ± 1.21	15.28 ± 0.78	0.803 ± 0.041
	B	267	497	19.88 ± 0.60	10.68 ± 0.65	0.537 ± 0.033
Cacodylic acid (μg/ml)						
25	A	436	482	19.28 ± 1.14	17.44 ± 0.84	0.905 ± 0.043
	B	288	498	19.92 ± 0.81	11.52 ± 0.68	0.578 ± 0.034
50	A	367	474	18.96 ± 1.31	14.68 ± 0.77	0.774 ± 0.040
	B	298	496	19.84 ± 0.69	11.92 ± 0.69	0.601 ± 0.035
100	A	404	472	18.88 ± 0.93	16.16 ± 0.80	0.856 ± 0.043
	B	292	492	19.68 ± 0.95	11.80 ± 0.69	0.600 ± 0.035
200	A	330	472	18.88 ± 1.30	13.20 ± 0.73	0.699 ± 0.038
	B	300	492	19.68 ± 0.80	12.00 ± 0.69	0.610 ± 0.035
400	A	274	487	19.48 ± 1.56	10.96 ± 0.66	0.563 ± 0.034
	B	361	493	19.72 ± 1.14	14.44 ± 0.76	0.732 ± 0.039
Positive control (10^{-3} M ethyl methanesulfonate)	A	854	486	19.44 ± 1.50	34.16 ± 1.17	1.757 ± 0.060
	B	1064	500	20.00 ± 0.71	42.56 ± 1.31	2.128 ± 0.065

Table 97

SISTER CHROMATID EXCHANGE (SCE) FREQUENCIES IN CHINESE HAMSTER OVARY (CHO) CELLS EXPOSED TO CACODYLIC ACID FOR 21.5 HOURS WITHOUT METABOLIC ACTIVATION

Treatment	Sample No.	Cytogeneticist[*]	No. of SCEs	No. of Chromosomes	Chromosomes/Cell (Mean ± SD)	SCEs/Cell[†] (Mean ± SE)	SCEs/Chromosome[†] (Mean ± SE)
Negative control (0.95% ethanol)	1	A	382	476	19.04 ± 1.21	15.28 ± 0.78	0.803 ± 0.041
	1	C	332	491	19.64 ± 0.86	13.28 ± 0.73	0.676 ± 0.037
	2	B	267	497	19.88 ± 0.60	10.68 ± 0.65	0.537 ± 0.033
Cacodylic acid (µg/ml)							
25	3	A	436	482	19.28 ± 1.14	17.44 ± 0.84	0.905 ± 0.043
	3	C	283	498	19.92 ± 0.48	11.32 ± 0.67	0.568 ± 0.034
	4	B	288	498	19.92 ± 0.81	11.52 ± 0.68	0.578 ± 0.034
50	5	A	367	474	18.96 ± 1.31	14.68 ± 0.77	0.774 ± 0.040
	6	B	298	496	19.84 ± 0.69	11.92 ± 0.69	0.601 ± 0.035
	6	C	273	494	19.76 ± 0.60	10.92 ± 0.66	0.553 ± 0.033
100	7	A	404	472	18.88 ± 0.93	16.16 ± 0.80	0.856 ± 0.043
	8	B	292	492	19.68 ± 0.95	11.80 ± 0.69	0.600 ± 0.035
	8	C	322	503	20.12 ± 0.60	12.88 ± 0.72	0.640 ± 0.036
200	9	A	330	472	18.88 ± 1.30	13.20 ± 0.73	0.699 ± 0.038
	10	B	300	492	19.68 ± 0.80	12.00 ± 0.69	0.610 ± 0.035
	10	C	328	494	19.76 ± 0.66	13.12 ± 0.72	0.664 ± 0.036
400	11	A	274	487	19.48 ± 1.56	10.96 ± 0.66	0.563 ± 0.034
	11	C	316	490	19.60 ± 0.50	12.64 ± 0.71	0.645 ± 0.036
	12	B	361	493	19.72 ± 1.14	14.44 ± 0.76	0.732 ± 0.039
Positive control (10^{-3} M ethyl methanesulfonate)	13	A	854	486	19.44 ± 1.50	34.16 ± 1.17	1.757 ± 0.060
	14	B	1064	500	20.00 ± 0.71	42.56 ± 1.31	2.128 ± 0.065

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[*] Each cytogeneticist analyzed 25 cells per sample.

[†] A one-way analysis of variance comparing the SCE frequencies in CHO cells exposed to cacodylic acid and to the negative control indicated that the variance between treatment groups was not significantly greater than the variance within treatment groups.

Table 98

SISTER CHROMATID EXCHANGE (SCE) FREQUENCIES IN CHINESE HAMSTER OVARY (CHO) CELLS EXPOSED TO CACODYLIC ACID FOR 2 HOURS WITH METABOLIC ACTIVATION

Treatment	Cyto-genet-icist*	No. of SCEs	No. of Chromosomes	Chromosomes/Cell (Mean ± SD)	SCEs/Cell† (Mean ± SE)	SCEs/Chromosome† (Mean ± SE)
Negative control (0.95% ethanol)	A	526	469	18.76 ± 0.78	21.04 ± 0.92	1.122 ± 0.049
	B	479	489	19.56 ± 0.92	19.16 ± 0.88	0.980 ± 0.045
Cacodylic acid (µg/ml)						
187.5	A	507	477	19.08 ± 0.76	20.28 ± 0.90	1.063 ± 0.047
	B	514	483	19.32 ± 0.80	20.56 ± 0.91	1.064 ± 0.047
375	A	475	471	18.84 ± 0.80	19.00 ± 0.87	1.008 ± 0.046
	B	466	496	19.84 ± 0.90	18.64 ± 0.86	0.940 ± 0.044
750	A	530	483	19.32 ± 0.80	21.20 ± 0.92	1.097 ± 0.048
	B	513	484	19.36 ± 0.76	20.52 ± 0.91	1.060 ± 0.047
1500	A	502	481	19.24 ± 1.01	20.08 ± 0.90	1.044 ± 0.047
	B	566	494	19.76 ± 0.78	22.64 ± 0.95	1.146 ± 0.048
3000	--‡	--	--	--	--	--
Positive control (10^{-3} M dimethylnitrosamine)	A	898	477	19.08 ± 1.19	35.92 ± 1.20	1.883 ± 0.063
	B	1050	492	19.68 ± 0.80	42.00 ± 1.30	2.134 ± 0.066

---

[*] Each cytogeneticist analyzed 25 cells per sample.

[†] A one-way analysis of variance comparing the SCE frequencies in CHO cells exposed to cacodylic acid and to the negative control indicated that the variance between treatment groups was not significantly greater than the variance within treatment groups.

[‡] Insufficient M_2 metaphase cells for analysis.

Table 99

MOUSE LYMPHOMA FORWARD MUTATION CONCENTRATION-RANGE ASSAY OF CACODYLIC ACID IN THE PRESENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (µg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency ($\times 10^{-6}$)	Induced Mutation Frequency ($\times 10^{-6}$)
Solvent control (1% H_2O)	93.7	600	448	178	102.2	95.7	79	--
	106.3	600	429	159	97.8	104.0	74	--
Positive control (5 µg/ml 3-MCA)	75.0	600	292	444	66.6	49.9	304	227
	67.5	600	298	447	68.0	45.9	300	223
Cacodylic acid								
1	106.3	600	493	166	112.4	119.5	67	--
2	77.7	600	543	194	123.8	96.2	71	--
5	104.7	600	567	213	129.3	135.4	75	--
10	111.2	600	489	180	111.5	124.0	74	--
20	109.9	600	561	203	127.9	140.6	72	--
40	94.5	600	585	187	133.4	126.1	64	--
80	104.7	600	--*	162	--	--	--	--
100	93.7	600	561	147	127.9	119.9	52	--
150	94.5	600	578	204	131.8	124.6	71	--
200	81.0	600	480	122	109.5	88.7	51	--
300	100.1	600	543	205	123.8	124.0	76	--
400	50.3	600	501	194	114.3	57.5	77	--
500	86.9	600	473	237	107.9	93.7	100	23
600	73.6	600	527	190	120.2	88.5	72	--
700	65.1	600	482	294	109.9	71.6	122	45
800	61.4	600	466	296	106.3	65.3	127	50
900	44.6	600	403	336	91.9	41.0	167	90
1000	41.9	600	363	301	82.8	34.7	166	89

---

*Sample lost.

Table 100

MOUSE LYMPHOMA FORWARD MUTATION ASSAY OF CACODYLIC ACID IN THE ABSENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency ($\times 10^{-6}$)	Induced Mutation Frequency ($\times 10^{-6}$)
Solvent control (1% H_2O)	97.7	600	575	104	96.4	94.2	36	--
	102.3	600	618	92	103.6	106.0	30	--
Positive control (500 μg/ml EMS)	65.5	600	406	1128	68.1	44.6	556	523
	63.0	600	376	1168	63.0	39.7	621	588
Cacodylic acid								
150	93.2	600	592	81	99.2	92.5	27	--
	88.9	600	519	93	87.0	77.3	36	3
200	80.9	600	502	90	84.2	68.1	36	3
	84.9	600	523	79	87.7	74.4	30	--
300	74.2	600	514	98	86.2	63.9	38	5
	74.2	600	534	158	89.5	66.4	59	26
400	68.6	600	495	124	83.0	56.9	50	17
	65.8	600	492	88	82.5	54.3	36	3
500	63.6	600	442	110	74.1	47.3	50	17
	59.0	600	502	124	84.2	49.7	49	16
600	52.8	600	--*	198	--	--	--	--
	51.3	600	383	140	64.2	32.9	73	40
700	49.8	600	436	101	73.1	36.4	46	13
	48.3	600	428	113	71.8	34.7	53	20
800	36.4	600	415	105	69.6	25.3	51	18
	41.6	600	431	103	72.3	30.1	48	15
900	19.3	600	407	165	68.2	13.2	81	48
	28.2	600	390	167	65.4	18.4	86	53
1000	21.4	600	310	177	52.0	11.1	114	81
	18.8	600	274	204	45.9	8.6	149	116

---

*Sample lost.

Table 101

REPEAT MOUSE LYMPHOMA FORWARD MUTATION ASSAY OF CACODYLIC ACID IN THE ABSENCE OF A METABOLIC SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency (× 10^{-6})	Induced Mutation Frequency (× 10^{-6})
Solvent control (1% H_2O)	104.6	600	470	186	105.7	110.6	79	--
	95.4	600	419	184	94.3	89.9	88	--
Positive control (500 μg/ml EMS)	65.7	600	312	1081	70.2	46.1	693	609
	65.7	600	302	979	67.9	44.6	648	564
Cacodylic acid								
105	83.3	600	465	110	104.6	87.1	47	--
	87.8	600	499	168	112.3	98.6	67	--
195	73.9	600	364	129	81.9	60.5	71	--
	73.5	600	417	100	93.8	69.0	48	--
300	63.5	600	350	129	78.7	50.0	74	--
	60.9	600	264	127	59.4	36.2	96	12
405	61.4	600	341	135	76.7	47.1	79	--
	53.4	600	406	136	91.3	48.8	67	--
495	55.5	600	378	110	85.0	47.2	58	--
	47.5	600	426	143	95.8	45.5	67	--
645	32.3	600	367	185	82.6	26.7	101	17
	33.6	600	350	229	78.7	26.5	131	47
795	29.4	600	316	269	71.1	20.9	170	86
	28.5	600	349	225	78.5	22.4	129	45
900	21.0	600	180	138	40.5	8.5	153	69
	16.8	600	282	307	63.4	10.7	218	134
1080	9.1	600	227	384	51.1	4.6	338	254
	9.8	600	119	321	44.8	4.4	323	239
1200	6.2	600	176	497	39.6	2.5	565	481
	6.7	600	172	401	38.7	2.6	466	382

Table 102

MOUSE LYMPHOMA FORWARD MUTATION ASSAY OF CACODYLIC ACID IN THE PRESENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency ($\times 10^{-6}$)	Induced Mutation Frequency ($\times 10^{-6}$)
Solvent control (1% H_2O)	100.8	600	543	117	100.6	101.5	43	--
	99.2	600	536	146	99.4	98.6	54	--
Positive control (5 μg/ml 3-MCA)	56.0	600	388	557	71.9	40.3	287	238
	53.7	600	395	559	73.2	39.3	283	234
Cacodylic acid								
420	89.2	600	554	153	102.7	91.6	55	6
	86.4	600	573	146	106.2	91.8	51	2
490	89.2	600	542	176	100.5	89.6	65	16
	85.2	600	534	196	99.0	84.3	73	24
630	81.4	600	550	156	101.9	83.0	57	8
	83.9	600	553	168	102.5	86.0	61	12
770	74.0	600	501	160	92.9	68.7	64	15
	30.4	600	484	176	89.7	27.3	73	24
910	71.8	600	445	221	82.5	59.2	99	50
	76.4	600	439	200	81.4	62.2	91	42
980	67.9	600	430	199	79.7	54.1	93	43
	69.1	600	417	190	77.3	53.4	91	42
1050	67.9	600	475	213	88.0	59.8	90	41
	66.0	600	--*	199	--	--	--	--
1190	65.7	600	461	260	85.4	56.1	113	64
	59.5	600	406	257	75.3	44.8	127	78
1330	54.5	600	471	267	87.3	47.6	113	64
	53.1	600	347	257	64.3	34.2	148	99
1400	53.2	600	367	320	68.0	36.2	174	125
	53.1	600	319	317	59.1	31.4	199	150

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*Sample lost.

Table 103

DOSING AND SACRIFICE SCHEDULE

Compound	No. Animals	0 Hr	24 Hr	Mean Body Wt Change (g)	Number Sacrificed 48 Hr	72 Hr	96 Hr	No. Fatalities
HBSS	24	5 ml/kg	10 ml/kg	+ 1.0	8	8	8	0
Cacodylic Acid								
Low dose	24	188 mg/kg	375 mg/kg	+1.0	8	8	7	1
Medium dose	24	375 mg/kg	750 mg/kg	+0.9	8	8	8	0
High dose	24	750 mg/kg	1500 mg/kg	+0.4	6	5	0	13

Table 104

INCIDENCE OF MICRONUCLEATED POLYCHROMATOPHILIC ERYTHROCYTES*
(500 PCEs Scored per Animal)

Dose	48 Hours Animal No.	48 Hours PCEs with MN	48 Hours PCE/RBC	72 Hours Animal No.	72 Hours PCEs with MN	72 Hours PCE/RBC	96 Hours Animal No.	96 Hours PCEs with MN	96 Hours PCE/RBC
Vehicle control	4ø	1	1.18	6ø	1	1.27	5ø	1	1.87
	4r	3	1.15	6r	2	1.32	5r	1	1.24
	4ℓ	1	2.10	6ℓ	0	0.93	5ℓ	2	0.57
	4b	0	0.86	6b	2	1.30	5b	1	2.13
	7ø	1	0.92	8ø	1	1.14	45ø	0	1.11
	7r	1	0.94	8r	0	0.58	45r	0	0.60
	7ℓ	0	1.43	8ℓ	2	0.88	45ℓ	2	1.69
	7b	1	0.97	8b	1	1.14	45b	1	0.79
	Total	8			9			7	
	Average		1.19			1.07			1.25
Cacodylic Acid 188 + 375 mg/kg	10ø	1	1.15	11ø	2	0.59	9ø	3	1.26
	10r	1	1.20	11r	2	1.00	9ℓ	3	2.15
	10ℓ	0	0.91	11ℓ	3	0.97	9b	0	0.79
	10b	1	0.62	11b	4	1.09	14ø	3	1.33
	12ø	1	1.45	13ø	2	1.36	14r	1	1.04
	12r	4	1.03	13r	0	2.25	14ℓ	3	1.08
	12ℓ	2	1.23	13ℓ	2	1.00	14b	1	1.37
	12b	0	1.04	13b	2	0.51			
	Total	10			17			14	
	Average		1.08			1.10			1.29
Cacodylic Acid 375 + 750 mg/kg	20ø	1	1.00	15ø	3	1.00	16ø	0	1.05
	20r	0	1.06	15r	3	1.04	16r	2	1.74
	20ℓ	2	0.68	15ℓ	3	0.95	16ℓ	3	1.23
	20b	1	0.91	15b	2	0.51	16b	2	1.35
	17r	2	0.81	18ø	1	1.16	19ø	2	1.82
	17ℓ	1	1.07	18r	2	0.63	19r	1	1.06
	17b	0	1.20	18ℓ	1	1.44	19ℓ	1	2.44
	17ø	3	1.20	18b	2	0.72	19b	2	1.00
	Total	10			17			13	
	Average		0.99			0.93			1.34
Cacodylic Acid 750 + 1500 mg/kg	22ℓ	5	0.69	24ø	5	1.17			
	23ℓ	1	2.47	24b	3	0.88			
	25ø	4	1.10	26ø	2	1.10			
	25r	2	1.12	26ℓ	3	1.27			
	25ℓ	8	0.88	26b	3	0.38			
	25b	2	1.30						
	Total	22			16				
	Average		1.26			0.96			

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*PCE = polychromatophilic erythrocyte
RBC = mature erythrocyte
MN = micronuclei
HBSS = Hank's balanced salt solution

Captan

S. typhimiurium Plate Incorporation (Table 105). Captan was previously tested in strains TA100, TA1535, TA1537, and TA1538 (51). It was found to be positive with and without activation in strains TA100 and TA1535, but negative in strains TA1537 and TA1538. It was tested at higher doses in strains TA98, TA100, and TA1538 under the present contract and was positive in TA98 and TA100 both with and without activation. The results for strain TA1538 were inconclusive due to toxicity.

E. coli WP-2. This compound was previously tested and found to be positive with and without activation (51).

S. cerevisiae D3 (Table 106). Captan was previously tested at a single dose and found to be positive with and without activation (51). It was retested over a range of doses under the present contract, with similar findings.

E. coli/B. subtilis Relative Toxicity. This compound was previously tested in this system and was positive in both species (51).

S. typhimurium Relative Toxicity (Tables 107, 108). Captan was positive in both strain pairs.

L5178Y Mouse Lymphoma (Tables 109-112). The initial concentration-range assay of captan was performed with 18 concentrations between 1 and 1000 μg/ml. The samples exposed in the absence of the metabolic activation system showed extreme cytotoxicity at all test concentrations, with relative suspension growth values never rising above 0.9%. Therefore, this concentration-range assay was repeated with 18 concentrations between 0.004 and 50 μg/ml (Table 109). The results showed that the approximate range to use in the mutagenesis assay was between 0.08 and 0.3 μg/ml, based on relative total growth values of 91.2% and 13.6%, respectively. Table 110 presents the results of concentration-range testing of captan in the presence of the metabolic activation system. Extreme cytotoxicity was observed at test concentrations of 40 μg/ml and higher, with relative suspension growth values of less than 5.0%. The results showed that the approximate range to use in the mutagenesis assay was 1 to 20 μg/ml, based on relative total growth values of 105.8% and 28.5%, respectively.

Table 111 presents the results of mutagenesis testing in the absence of the metabolic activation system. Captan was extremely cytotoxic at test concentrations greater than 0.5 μg/ml, yielding relative suspension growth values of less than 3.0%. No significant depression in the relative total growth was observed at the lowest concentration tested (0.04 μg/ml), which yielded an average relative total growth value of 95.4%. From 0.4 to 0.5 μg/ml, a concentration-related increase in induced mutation frequency was observed. Mutation frequencies of at least twice the spontaneous frequency were observed at 0.403 μg/ml; relative growth values at this concentration were over 10.0%.

Table 112 presents the results of mutagenesis testing in the presence of the metabolic activation system. The highest tested concentration of 26 μg/ml yielded an average relative total growth value of 7.5%. No significant depression in the relative total growth value was observed at or above 3 μg/ml, which yielded an average total growth value of 98.9%. From 3 to 26 μg/ml, a concentration-related increase in induced mutation frequency was observed. Average mutation frequencies at least twice the spontaneous frequency of the acceptable solvent control sample were observed at 22 μg/ml and 20 μg/ml; the average relative total growth values at these concentrations were 18.0% and 32.4%, respectively.

These results indicate a mutagenic effect of captan at the thymidine kinase locus in L5178Y mouse lymphoma cells both with and without the metabolic activation system. In both cases, captan increased the mutation frequencies, in a concentration-related manner, above the spontaneous frequencies observed in the controls. The mutagenic data obtained from the concentration-range assays in the absence and presence of the metabolic activation system further support the finding of a mutagenic effect of captan. The presence of the metabolic activation system was not required to elicit the mutagenic effect, but it did appear to decrease the cytotoxicity of captan.

UDS in WI-38 Cells (Tables 113-115). Captan was previously tested and reported as negative without activation, positive with activation (51). Since the control values for the previous test were unusually low, it was retested under the present contract. The control values were acceptable, and captan was found to be negative, both with and without activation.

Mouse Dominant Lethal Test. Captan was previously tested and found negative in this system (51).

Table 105

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

CAPTAN
Retest

Compound	Metabolic Activation	Compound Added (µg/plate)	Histidine Revertants per Plate TA1535	TA1537	TA1538	TA98	TA100
Negative Control							
DMSO	-				25 16	28 30	135 138
	+				23 25	28 38	139 132
Positive Controls:							
2-Anthramine	-	1.0			52	42	145
	+	1.0			310	324	746
Sodium Azide	-	0.5					397
2-Nitrofluorene	-	5.0			817	543	
Captan	-	25.0			42	198	492
	-	50.0			6T	27T	25T
	-	75.0			T	T	T
	-	100.0			T	T	T
	-	150.0			T	T	T
	-	200.0			T	T	T
	+	25.0			63	257	933
	+	50.0			61	211	737
	+	75.0			38	247	520
	+	100.0			18T	203	96T
	+	150.0			T	T	T
	+	200.0			T	T	T

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* T, toxic.

Table 106

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

CAPTAN

Retest

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($x 10^{-7}$)	Percent	Per ml ($x 10^{-3}$)	Per 10^5 Survivors
Negative Control: DMSO	-		8.5	100	6.5	7.7
	+		7.7	100	3.0	3.9
Positive Control 1,2,3,4-Diepoxybutane	-	0.025	8.9	105	941.3	1057.6
Captan	-	0.001	9.9	116	26.0	26.3
	-	0.002	8.9	105	51.0	57.3
	-	0.003	7.2	85	56.0	77.8
	-	0.004	6.7	79	61.0	91.0
	-	0.005	T*	T	T	T
	+	0.001	9.2	119	19.0	20.7
	+	0.002	6.6	86	38.0	57.9
	+	0.003	7.2	94	35.0	48.6
	+	0.004	8.9	116	29.0	32.6
	+	0.005	6.9	90	45.0	65.2

* T, toxic.

Table 107

DIFFERENTIAL TOXICITY ASSAYS WITH SALMONELLA TYPHIMURIUM STRAINS

Experiment 1

Compound	Amount of Compound Applied to Disc	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		SL4525 (rec$^+$)		SL4700 (rec$^-$)		TA1978 (uvrB$^+$)		TA1538 (uvrB$^-$)	
Negative Control									
Kanamycin	100.0 μg	**14**	**13**	**13**	**14**	**13**	**13**	**14**	**14**
Positive Controls									
MMS[*]	**10.0 mg**	**47**	**46**	**68**	**65**				
4-NQO[†]	**2.0 mg**					**12**	**12**	**24**	**23**
Captan	0.1 mg	14	13	19	19	13	14	21	21
	0.5 mg	15	14	21	19	15	16	23	22

Table 108

DIFFERENTIAL TOXICITY ASSAYS WITH SALMONELLA TYPHIMURIUM STRAINS

Experiment 2

Compound	Amount of Compound Applied to Disc	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		SL4525 (rec^+)		SL4700 (rec^-)		TA1978 ($uvrB^+$)		TA1538 ($uvrB^-$)	
Negative Control									
Kanamycin	100.0 µg	15	14	16	15	17	17	15	16
Positive Controls									
MMS*	10.0 mg	45	40	72	74				
4-NQO†	2.0 mg					12	12	28	26
Captan	0.1 mg	18	18	24	21	14	15	23	24
	0.5 mg	20	19	23	24	16	16	23	24

Table 109

MOUSE LYMPHOMA FORWARD MUTATION CONCENTRATION-RANGE ASSAY OF CAPTAN IN THE ABSENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (µg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency (× 10⁻⁶)	Induced Mutation Frequency (× 10⁻⁶)
Solvent control (1% DMSO)	92.7	600	543	115	100.7	93.4	42	--
	107.3	600	535	77	99.3	106.5	29	--
Positive control (500 µg/ml EMS)	81.0	600	310	890	57.5	46.6	574	538
	71.2	600	323	947	59.9	42.7	586	550
Captan								
0.004	97.7	600	498	105	92.4	90.3	42	6
0.0064	91.3	600	442	156	82.0	74.9	71	35
0.008	97.7	600	432	121	80.1	78.3	56	20
0.012	93.7	600	502	171	93.1	87.3	68	32
0.02	89.9	600	466	139	86.5	77.7	60	24
0.04	88.6	600	537	158	99.6	88.3	59	23
0.06	97.0	600	514	162	95.4	92.5	63	27
0.08	92.7	600	530	142	98.3	91.2	54	18
0.10	77.4	600	414	149	76.8	59.5	72	36
0.20	41.4	600	438	169	81.3	33.6	77	41
0.30	17.9	600	410	290	76.1	13.6	141	105
0.50	3.8	600	375	466	69.6	2.6	249	213
0.65	2.8	NC*	--	--	--	--	--	--
0.80	1.3	NC	--	--	--	--	--	--
1	0.5	NC	--	--	--	--	--	--
10	0.4	NC	--	--	--	--	--	--
20	0.5	NC	--	--	--	--	--	--
50	0.6	NC	--	--	--	--	--	--

---

*Not cloned.

Table 110

MOUSE LYMPHOMA FORWARD MUTATION CONCENTRATION-RANGE ASSAY OF CAPTAN IN THE PRESENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency ($\times 10^{-6}$)	Induced Mutation Frequency ($\times 10^{-6}$)
Solvent control (1% DMSO)	98.6	600	537	258	96.2	94.8	96	--
	101.4	600	580	229	103.8	105.3	79	--
Positive control (5 μg/ml 3-MCA)	39.2	600	261	548	46.7	18.3	420	332
	64.8	600	415	614	74.3	48.2	296	208
Captan								
1	98.0	600	603	295	108.0	105.8	98	10
2	101.4	600	489	274	87.6	88.8	112	24
5	84.7	600	571	309	102.2	86.6	108	20
10	79.5	600	364	270	65.2	51.8	148	60
20	32.0	600	498	459	89.2	28.5	184	96
40	2.5	NC*	--	--	--	--	--	--
80	0.5	NC	--	--	--	--	--	--
100	1.3	NC	--	--	--	--	--	--
150	2.0	NC	--	--	--	--	--	--
200	4.6	NC	--	--	--	--	--	--
300	PPT†	--	--	--	--	--	--	--
400	PPT	--	--	--	--	--	--	--
500	PPT	--	--	--	--	--	--	--
600	PPT	--	--	--	--	--	--	--
700	PPT	--	--	--	--	--	--	--
800	PPT	--	--	--	--	--	--	--
900	PPT	--	--	--	--	--	--	--
1000	PPT	--	--	--	--	--	--	--

---

*Not cloned.

†Persistent precipitate present after washdown procedure; sample discarded.

Table III

MOUSE LYMPHOMA FORWARD MUTATION ASSAY OF CAPTAN IN THE ABSENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (µg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency (× 10⁻⁶)	Induced Mutation Frequency (× 10⁻⁶)
Solvent control (1% DMSO)	97.9	600	575	264	103.8	101.6	92	--
	102.1	600	533	261	96.2	98.2	98	--
Positive control (500 µg/ml EMS)	63.7	600	264	1009	47.7	30.4	764	669
	66.2	600	274	1018	49.5	32.7	743	648
Captan								
0.04	102.1	600	513	286	92.6	94.5	112	17
	96.9	600	550	392	99.3	96.2	143	48
0.06	96.8	600	--*	331*	--	--	--	--
	92.5	600	--*	--	--	--	--	--
0.086	81.1	600	511	353	92.2	74.8	138	43
	88.1	600	494	268	89.2	78.6	109	14
0.098	77.6	600	485	308	87.5	67.9	127	32
	80.8	600	413	312	74.5	60.2	151	56
0.15	59.3	600	432	360	78.0	46.2	167	72
	61.3	600	443	350	80.0	49.0	158	63
0.195	46.1	600	415	387	74.9	34.5	187	92
	43.1	600	533	365	96.2	41.5	137	42
0.30	26.5	600	428	359	77.3	20.5	168	73
	26.5	600	434	422	78.3	20.8	194	99
0.403	14.7	600	443	487	80.0	11.8	220	125
	14.9	600	421	493	76.0	11.3	234	139
0.50	7.0	600	347	547	62.6	4.4	315	220
	7.7	600	329	519	59.4	4.6	316	221
0.65	2.3	NC+	--	--	--	--	--	--
	1.4	NC	--	--	--	--	--	--

---

*Sample lost.

+Not cloned.

Table 112

MOUSE LYMPHOMA FORWARD MUTATION ASSAY OF CAPTAN IN THE PRESENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency (× 10^{-6})	Induced Mutation Frequency (× 10^{-6})
Solvent control (1% DMSO)	101.4 98.6	600 600	580 482	271 312	109.2 90.8	110.8 89.5	93 129*	-- --
Positive control (6 μg/ml 3-MCA)	40.0 50.3	600 600	308 458	666 707	58.0 86.3	23.2 43.4	432 309	339 216
Captan								
0.3	101.9 104.8	600 600	595 477	292 303	112.1 89.8	114.2 94.1	98 127	5 34
0.9	101.9 101.9	600 600	587 505	314 335	110.5 95.1	112.6 96.9	107 133	14 40
3	99.7 104.8	600 600	540 491	306 347	101.7 92.5	101.4 96.9	113 141	20 48
6	89.7 92.5	600 600	518 548	365 339	97.6 103.2	87.5 95.5	141 124	48 31
12	72.3 80.3	600 600	620 596	488 451	116.8 112.2	84.4 90.1	157 151	64 58
14	72.6 63.6	600 600	--† 620	458 443	-- 116.8	-- 74.3	-- 143	-- 50
17	58.9 65.2	600 600	561 573	476 395	105.6 107.9	62.2 70.4	170 138	77 45
20	27.1 36.9	600 600	500 565	482 538	94.2 106.4	25.5 39.3	193 190	100 97
22	26.0 17.3	600 600	456 420	463 489	85.9 79.1	22.3 13.7	203 233	110 140
26	9.9 12.4	600 600	366 353	515 470	68.9 66.5	6.8 8.2	281 266	188 173

*The spontaneous mutation frequency of one solvent control sample is not acceptable as it exceeds the assay standard of 100 x 10^{-6}. However, this assay was included in the evaluation of captan because the spontaneous frequency of the other control was below 100 x 10^{-6} and the other criteria for a valid assay were met.

†Sample lost.

Table 113

UNSCHEDULED DNA SYNTHESIS ASSAY OF CAPTAN WITHOUT ACTIVATION
(dpm/μg DNA)
Retest

	Concentration of Compounds Tested						
	Captan (M x 10^{-6})						4NQO (M)
Sample	0*	3.1	6.3	12.5	25	50	10^{-5}
1	45	50	42	19	9	3	1295
2	54	58	55	30	9	0	1526
3	59	44	52	28	8	3	1487
4	59	43	53	31	9	5	1397
5	61	59	37	24	6	0	1418
6	54	55	--†	40	3	6	561
Mean	55	52	48	29	8	3	1281
SD	6	7	8	7	2	3	362
SE	3	3	4	3	1	1	148

---

[*] Negative control and compound solvent, 0.5% DMSO.

[†] Sample lost.

Table 114

UNSCHEDULED DNA SYNTHESIS ASSAY OF CAPTAN WITH METABOLIC ACTIVATION
(dpm/μg DNA)

Retest 1

	Concentration of Compounds Tested						
	Captan (M x 10^{-6})						DMN (M)
Sample	0*	3.7	11.1	33.3	100	300	5 x 10^{-2}
1	126	179	151	165	138	178	471
2	123	142	123	153	118	144	270
3	129	151	131	154	127	125	164
4	90	182	124	183	93	123	480
5	102	157	121	144	100	148	416
6	144	151	126	147	73	128	518
Mean	119	161	130	158	108	141	386
SD	20	16	11	14	24	21	139
SE	8	7	5	6	10	8	57

---

*Negative control and compound solvent, 0.5% DMSO.

Table 115

UNSCHEDULED DNA SYNTHESIS ASSAY OF CAPTAN WITH METABOLIC ACTIVATION

(dpm/μg DNA)

Retest 2

Sample	Concentration of Compounds Tested Captan (M x 10-6) 0*	 12.3	 37	 111	 333	 1000†	 DMN (M) 5 x 10^{-2}
1	48	60	58	76	44	65	266
2	62	68	75	72	53	86	326
3	33	59	72	66	76	40	236
4	71	81	95	77	44	66	238
5	86	78	66	80	90	61	287
6	96	83	75	57	60	68	312
Mean	66	72	73	71	61	64	277
SD	24	10	12	8	18	14	38
SE	10	4	5	3	8	6	15

---

[*] Negative control and compound solvent, 0.5% DMSO.

[†] Precipitate observed at 1 x 10^{-3} M.

Chlorpyrifos

S. typhimurium Plate Incorporation (Table 116). Chlorpyrifos was previously tested in strains TA100, TA1535, TA1537 and TA1538 (51). It was found to be negative, both with and without activation, in all four strains. It was retested at higher doses in strains TA98 and TA100, and was negative, both with and without activation in both strains.

E. coli WP-2, S. cerevisiae D3. This compound was previously tested in both systems and was negative, with and without activation (51).

E. coli/B. subtilis Relative Toxicity. Chlorpyrifos was positive in both species in a previous test (51).

S. typhimurium Relative Toxicity (Tables 117, 118). This compound was positive in both strain pairs.

UDS in WI-38 Cells. Chlorpyrifos was negative, both with and without activation, in previous tests (51).

Table 116

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

CHLOROPYRIFOS
Retest

Compound	Metabolic Activation	Compound Added (µg/plate)	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative Control							
DMSO	-				25 16	28 30	135 138
	+				23 25	28 38	139 132
Positive Controls:							
2-Anthramine	-	1.0			52	42	145
	+	1.0			310	324	746
Sodium Azide	-	0.5					397
2-Nitrofluorene	-	5.0			817	543	
Chloropyrifos	-	500.0				26	111
	-	1,000.0				29	119
	-	2,000.0				20	100
	-	3,000.0				30	115
	-	4,000.0				13	125
	-	5,000.0				15	102
	+	500.0				31	120
	+	1,000.0				28	131
	+	2,000.0				34	107
	+	3,000.0				29	106
	+	4,000.0				37	139
	+	5,000.0				34	129

Table 117

DIFFERENTIAL TOXICITY ASSAYS WITH SALMONELLA TYPHIMURIUM STRAINS

Experiment 1

Compound	Amount of Compound Applied to Disc	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		SL4525 (rec^+)		SL4700 (rec^-)		TA1978 ($uvrB^+$)		TA1538 ($uvrB^-$)	
Negative Control									
Kanamycin	100.0 μg	14	13	13	14	13	13	14	14
Positive Controls									
MMS[*]	10.0 mg	47	46	68	65				
4-NQO[†]	2.0 mg					12	12	24	23
Chlorpyrifos	1.0 mg	7	7	7	7	7	7	7	7
	5.0 mg	8	10	13	14	10	10	14	14

Table 118

DIFFERENTIAL TOXICITY ASSAYS WITH SALMONELLA TYPHIMURIUM STRAINS

Experiment 2

Compound	Amount of Compound Applied to Disc	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		SL4525 (rec$^+$)		SL4700 (rec$^-$)		TA1978 (uvrB$^+$)		TA1538 (uvrB$^-$)	
Negative Control									
Kanamycin	100.0 µg	15	14	16	15	17	17	15	16
Positive Controls									
MMS*	10.0 mg	45	40	72	74				
4-NQO†	2.0 mg					12	12	28	26
Chlorpyrifos	5.0 mg	12	12	13	12	8	8	20	19
	10.0 mg	19	18	21	22	10	11	20	21

Crotoxyphos

S. typhimurium Plate Incorporation, E. coli WP-2. This compound was previously tested and found to be negative in both of these test systems with and without activation (52).

S. cerevisiae D3. Crotoxyphos was found to be positive, both with and without activation, in a previous test (52).

S. cerevisiae D7 (Tables 119, 120). This pesticide was first tested over a concentration range of 0.005 to 0.05%, then at concentrations of 0.005 to 0.03%. No reproducible increase in mitotic crossing-over, gene conversion, or reverse mutation was observed with crotoxyphos.

E. coli/B. subtilis Relative Toxicity. Crotoxyphos was previously tested and reported to be negative in both species (52). Reexamination of the data indicates that the results are more appropriately regarded as inconclusive.

S. typhimurium Relative Toxicity (Tables 121, 122). This compound was negative in both strain pairs in this system.

SCE in CHO Cells (Tables 123-126). Crotoxyphos was dissolved in DMSO at a concentration of 0.5% for cytotoxicity testing with and without metabolic activation. Table 123 presents the results of the cytotoxicity test of crotoxyphos in CHO cell cultures without metabolic activation. At concentrations of 0.02% and greater, crotoxyphos was cytotoxic to CHO cells. At 0.004% or less, M_2 metaphase cells were produced. Table 124 presents the results of the cytotoxicity test of crotoxyphos in CHO cell cultures with metabolic activation. At a concentration of 0.1% and greater, crotoxyphos was cytotoxic to CHO cells. At 0.02% or less, M_2 metaphase cells were produced.

Table 125 presents the SCE frequencies in CHO cells exposed to crotoxyphos without metabolic activation. Cultures exposed to 0.008% crotoxyphos were not harvested because of excessive cytotoxicity. Cultures exposed to 0.004% crotoxyphos produced an insufficient number of second division metaphase cells for analysis. A one-way analysis of variance was conducted on SCE frequencies of the lower three concentrations of crotoxyphos and of the negative controls. The variance between treatment groups was not significantly greater than the variance within treatment groups.

Table 126 presents the SCE frequencies in CHO cells exposed to crotoxyphos with metabolic activation. A one-way analysis of variance was conducted on SCE frequencies of the five concentrations of crotoxyphos and of the negative controls. The variance between treatment groups was not significantly greater than the variance within treatment groups.

We conclude that, under the conditions of these experiments, crotoxyphos did not induce SCE in CHO cells, either with or without metabolic activation.

L5178Y Mouse Lymphoma (Tables 127-130). Table 127 presents the results of concentration-range testing of crotoxyphos in the absence of the metabolic activation system. The results showed that the range to use in mutagenesis tests was approximately 5 to 150 μg/ml (94.4% relative total growth at 5 μg/ml and 17.4% relative total growth at 150 μg/ml). Concentration-range test results in the presence of the metabolic activation system (Table 128) showed that the approximate range to use in the mutagenesis assay was 2 to 80 μg/ml (94.1% relative total growth at 2 μg/ml and 7.0% relative total growth at 80 μg/ml).

Table 129 presents the results of mutagenesis testing of crotoxyphos in the absence of the metabolic activation system. At the high concentrations of 200 μg/ml, the relative total growth was 4.0% (one duplicate was not cloned because the suspension growth was too low). No significant depression in relative total growth was observed at or below 30 μg/ml, which yielded an average relative total growth value of 96.8%. Over the range tested, a concentration-related increase in induced mutation frequency was observed. Mutation frequencies at least twice the spontaneous frequencies were observed from 150 to 180 μg/ml (four concentrations), with relative total growth values of over 10.0%.

Table 130 presents the results of mutagenesis testing of crotoxyphos in the presence of the metabolic activation system. At the high concentration (80 μg/ml), the average relative total growth was 6.8%. No significant depression in the relative total growth values was observed at 5 μg/ml, which yielded an average relative total growth value of 107.7%. Over the range tested, there was evidence of a concentration-related increase in induced mutation frequency. Average mutation frequencies of at least twice the spontaneous frequency of the acceptable solvent control sample were observed from 5 to 55 μg/ml (eight concentrations), with relative total growth values of over 10.0%.

These results indicate a mutagenic effect of crotoxyphos at the thymidine kinase locus in L5178Y mouse lymphoma cells both with and without the metabolic activation system. In both cases, crotoxyphos increased the mutation frequencies, in a concentration-related manner, above the spontaneous frequencies observed in controls. The presence of the metabolic activation system was not required to elicit the mutagenic effect, but it appeared to increase both the cytotoxicity and mutagenicity of crotoxyphos.

UDS in WI-38 Cells. Crotoxyphos was previously evaluated in this system, and was found to be negative, both with and without activation (52).

Micronucleus Test (Tables 131, 132). The dosage and sacrifice schedules are shown in Table 131. The incidences of micronucleated PCEs in blood and bone marrow and the ratio of PCEs to mature red cells (RBCs) are given in Table 132. The results for all vehicle control and crotoxyphos-treated groups were negative ($\beta < 0.05$); the positive control TMP-treated groups were positive ($\alpha < 0.05$). The mean ratios of PCEs to RBCs were within the historical control range (0.5-1.5) for all dose-time groups.

The values obtained for the blood smears of treated animals were within the negative decision limits, although the significance of that negative decision was generally less than that for the bone marrow smears. The vehicle control and compound-treated group totals were within the normal range for micronucleated cells in bone marrow, and the 30- and 72-hour TMP groups were positive. The negative result obtained in the 48-hour TMP group is thought to be a chance anomaly. The fatalities in the medium- and high-dose groups indicate that a maximum tolerated dose was administered.

In this micronucleus test, a sufficient number of cells and time points were examined at each dose level, the maximum possible dose was given, and the PCE-RBC ratio was not depressed. The results for all treatment groups were negative. Crotoxyphos is therefore negative in the micronucleus test.

Table 119

IN VITRO ASSAYS WITH *SACCHAROMYCES CEREVISIAE* D7

CROTOXYPHOS

Experiment 1

			Survivors		Crossing-Over				Gene Conversion		Reverse Mutation	
					Mitotic Recombinants		Total Aberrants		Convertants		Revertants	
Compound	MA	% Conc.	Cells Per ml ($\times 10^{-7}$)	%	Per ml ($\times 10^{-3}$)	Per 10^{3} Survivors	Per ml ($\times 10^{-3}$)	Per 10^{3} Survivors	Per ml ($\times 10^{-1}$)	Per 10^{6} Survivors	Per ml ($\times 10^{0}$)	Per 10^{7} Survivors
Negative Control												
DMSO			4.4	100	2.5	5.7	12	27	70	16	19	4.3
			4.5	100	3.0	6.7	17	38	74	16	18	4.0
Positive Controls												
1,2,3,4-Diepoxybutane	-	0.006	3.4	77	78	230	580	1,700	1,300	380	530	160
	+	0.006	3.8	84	120	320	720	1,900	1,400	370	490	130
Crotoxyphos	-	0.005	5.1	120	5.0	9.8	12	24	91	18	16	3.1
	-	0.0075	4.6	100	2.0	4.4	20	43	88	19	30	6.5
	-	0.01	4.7	110	2.5	5.3	10	21	81	17	33	7.0
	-	0.025	3.4	77	2.0	5.9	13	38	73	21	18	5.3
	-	0.05	1.8	41	4.0	22	10	56	36	20	13	7.2
	+	0.005	5.1	110	2.0	3.9	17	33	73	14	31	6.1
	+	0.0075	4.8	110	4.0	8.3	19	40	77	16	21	4.4
	+	0.01	4.8	110	6.3	13	21	44	81	17	30	6.3
	+	0.025	5.0	110	5.0	10	24	48	77	15	37	7.4
	+	0.05	3.9	87	5.0	13	29	74	56	14	26	6.7

Table 120

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D7

CROTOXYPHOS

Experiment 2

			Survivors		Crossing-Over				Gene Conversion		Reverse Mutation	
					Mitotic Recombinants		Total Aberrants		Convertants		Revertants	
Compound	MA	% Conc.	Cells Per ml $(\times 10^{-7})$	%	Per ml $(\times 10^{-3})$	Per 10^5 Survivors	Per ml $(\times 10^{-3})$	Per 10^5 Survivors	Per ml $(\times 10^{-1})$	Per 10^6 Survivors	Per ml $(\times 10^{0})$	Per 10^7 Survivors
Negative Control												
DMSO	-		5.4	100	2.0	3.7	17	32	72	13	16	3.0
	+		6.1	100	1.0	1.6	10	16	57	9.3	11	1.8
Positive Control												
1,2,3,4-Diepoxybutane	-	0.006	4.7	87	59	130	460	980	1,200	260	110	23
	+	0.006	4.8	79	74	150	490	1,000	1,200	250	190	40
Crotoxyphos	-	0.005	6.0	110	1.0	1.7	14	23	70	12	1.0	0.2
	-	0.0075	5.7	110	0	0	4.0	7.0	77	14	16	2.8
	-	0.01	5.5	100	0	0	1.0	1.8	81	15	11	2.0
	-	0.02	6.0	110	0	0	7.0	12	87	15	10	1.7
	-	0.03	5.8	110	0	0	6.0	10	65	11	8.8	1.5
	+	0.005	5.4	89	1.0	1.9	7.0	13	50	9.3	22	4.1
	+	0.0075	5.3	87	2.0	3.8	16	30	64	12	11	2.1
	+	0.01	5.2	85	0	0	13	25	61	12	7.0	1.4
	+	0.02	6.1	100	0	0	10	16	65	11	14	2.3
	+	0.03	4.8	79	2.0	4.2	16	33	71	15	20	4.2

Table 121

DIFFERENTIAL TOXICITY ASSAYS WITH SALMONELLA TYPHIMURIUM STRAINS

Experiment 1

Compound	Amount of Compound Applied to Disc	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		SL4525 (rec$^+$)		SL4700 (rec$^-$)		TA1978 (uvrB$^+$)		TA1538 (uvrB$^-$)	
Negative Control									
Kanamycin	100.0 µg	14	13	13	14	13	13	14	14
Positive Controls									
MMS[*]	10.0 mg	47	46	68	65				
4-NQO[†]	2.0 mg					12	12	24	23
Crotoxyphos	5.0 µl	8	8	8	8	8	8	8	8
	10.0 µl	8	9	8	8	8	8	8	8

Table 122

DIFFERENTIAL TOXICITY ASSAYS WITH SALMONELLA TYPHIMURIUM STRAINS

Experiment 2

Compound	Amount of Compound Applied to Disc	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		SL4525 (rec^+)		SL4700 (rec^-)		TA1978 ($uvrB^+$)		TA1538 ($uvrB^-$)	
Negative Control									
Kanamycin	**100.0 µg**	**15**	**14**	**16**	**15**	**17**	**17**	**15**	**16**
Positive Controls									
MMS***	**10.0 mg**	**45**	**40**	**72**	**74**				
4-NQO†	**2.0 mg**					12	12	28	26
Crotoxyphos	10.0 µl	8	8	9	8	8	8	8	9
	20.0 µl	9	9	9	9	9	9	10	10

Table 123

CYTOTOXICITY TEST OF CHINESE HAMSTER OVARY CELLS EXPOSED TO CROTOXYPHOS FOR 21.5 HOURS WITHOUT METABOLIC ACTIVATION

Treatment	Number of Divisions in Bromodeoxyuridine (BrdU)*
Negative Control (1% DMSO)	M_2
Crotoxyphos	
0.0008%	M_2
0.004%	$M_2 > M_1$
0.02%	--†
0.1%	--†
0.5%	--†
Positive control (10^{-3} M ethyl methanesulfonate)	M_2

---

*M_1, one division in BrdU; M_2, two divisions in BrdU.

†Cultures were not harvested because of excessive cytotoxicity.

Table 124

CYTOTOXICITY TEST OF CHINESE HAMSTER OVARY CELLS EXPOSED TO CROTOXYPHOS FOR 2 HOURS WITH METABOLIC ACTIVATION

Treatment	Number of Divisions In Bromodeoxyuridine (BrdU)[*]
Negative control (1% DMSO)	M_2
Crotoxyphos	
0.0008%	M_2
0.004%	M_2
0.02%	M_2
0.1%	--[†]
0.5%	--[†]
Positive control (10^{-3} M dimethylnitrosamine)	M_2

---

[*] M_2, two divisions in BrdU

[†] Cultures were not harvested because of excessive cytotoxicity.

Table 125

SISTER CHROMATID EXCHANGE (SCE) FREQUENCIES IN CHINESE HAMSTER OVARY (CHO) CELLS
EXPOSED TO CROTOXYPHOS FOR 21.5 HOURS WITHOUT METABOLIC ACTIVATION

Treatment	Cyto-geneticist*	No. of SCEs	No. of Chromosomes	Chromosomes/Cell (Mean ± SD)	SCEs/Cell† (Mean ± SE)	SCEs/Chromosome† (Mean ± SE)
Negative control (1% DMSO)	A	236	487	19.48 ± 0.82	9.44 ± 0.61	0.485 ± 0.032
	B	305	489	19.56 ± 0.65	12.20 ± 0.70	0.624 ± 0.036
Crotoxyphos						
0.0005%	A	251	488	19.52 ± 1.00	10.04 ± 0.63	0.514 ± 0.032
	B	271	484	19.36 ± 0.86	10.84 ± 0.66	0.560 ± 0.034
0.001%	A	280	478	19.12 ± 0.44	11.20 ± 0.67	0.586 ± 0.035
	B	327	493	19.72 ± 0.68	13.08 ± 0.72	0.663 ± 0.037
0.002%	A	308	495	19.80 ± 0.96	12.32 ± 0.70	0.622 ± 0.035
	B	328	493	19.72 ± 0.54	13.12 ± 0.72	0.665 ± 0.037
0.004%	†	--	--	--	--	--
		--	--	--	--	--
0.008%	§	--	--	--	--	--
		--	--	--	--	--
Positive control (10^{-3}M ethyl methanesulfonate)	A	681	478	19.12 ± 1.24	27.24 ± 1.04	1.425 ± 0.055
	B	962	479	19.16 ± 0.80	38.48 ± 1.24	2.008 ± 0.065

---

[*] Each cytogeneticist analyzed 25 cells per sample.

[†] A one-way analysis of variance comparing the SCE frequencies in CHO cells exposed to crotoxyphos and to the negative control indicated that the variance between treatment groups was not significantly greater than the variance within treatment groups.

[‡] Insufficient M_2 metaphase cells for analysis.

[§] Cultures were not harvested because of excessive cytotoxicity.

Table 126

SISTER CHROMATID EXCHANGE (SCE) FREQUENCIES IN CHINESE HAMSTER OVARY (CHO) CELLS EXPOSED TO CROTOXYPHOS FOR 2 HOURS WITH METABOLIC ACTIVATION

Treatment	Cyto-geneticist*	No. of SCEs	No. of Chromosomes	Chromosomes/Cell (Mean ± SD)	SCEs/Cell† (Mean ± SE)	SCEs/Chromosome† (Mean ± SE)
Negative control (1% DMSO)	A	304	482	19.28 ± 1.10	12.16 ± 0.70	0.631 ± 0.036
	B	322	478	19.12 ± 0.67	12.88 ± 0.72	0.674 ± 0.038
Crotoxyphos						
0.00125%	A	346	488	19.52 ± 0.59	13.84 ± 0.74	0.709 ± 0.038
	B	389	475	19.00 ± 0.65	15.56 ± 0.79	0.819 ± 0.042
0.0025%	A	383	485	19.40 ± 0.71	15.32 ± 0.78	0.790 ± 0.040
	B	369	481	19.24 ± 0.60	14.76 ± 0.77	0.767 ± 0.040
0.005%	A	363	488	19.52 ± 0.51	14.52 ± 0.76	0.744 ± 0.039
	B	363	474	18.96 ± 0.73	14.52 ± 0.76	0.766 ± 0.040
0.01%	A	396	484	19.36 ± 0.64	15.84 ± 0.80	0.818 ± 0.041
	B	446	478	19.12 ± 0.60	17.84 ± 0.84	0.933 ± 0.044
0.02%	A	357	479	19.16 ± 0.75	14.28 ± 0.76	0.745 ± 0.039
	B	433	483	19.32 ± 0.69	17.32 ± 0.83	0.896 ± 0.043
Positive control (10^{-1} M dimethylnitrosamine)	A	792	496	19.84 ± 1.03	31.68 ± 1.13	1.597 ± 0.057
	B	788	478	19.12 ± 0.67	31.52 ± 1.12	1.649 ± 0.059

---

[*] Each cytogeneticist analyzed 25 cells per sample.

[†] A one-way analysis of variance comparing the SCE frequencies in CHO cells exposed to crotoxyphos and to the negative control indicated that the variance between treatment groups was not significantly greater than the variance within treatment groups.

Table 127

MOUSE LYMPHOMA FORWARD MUTATION CONCENTRATION-RANGE ASSAY OF CROTOXYPHOS IN THE ABSENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency (× 10^{-6})	Induced Mutation Frequency (× 10^{-6})
Solvent control (1% DMSO)	99.5	600	519	108	98.1	97.6	42	--
	100.5	600	539	106	101.9	102.4	39	--
Positive control (500 μg/ml EMS)	65.1	600	320	1004	60.5	39.4	628	587
	60.6	600	319	969	60.3	36.5	608	567
Crotoxyphos								
1	95.4	600	486	99	91.9	87.6	41	--
2	96.3	600	485	106	91.7	88.3	44	3
5	90.8	600	550	170	104.0	94.4	62	21
10	95.4	600	466	95	88.1	84.0	41	--
20	90.8	600	475	133	89.8	81.5	56	15
40	82.9	600	519	162	98.1	81.3	62	21
80	60.6	600	482	113	91.1	55.2	47	6
100	53.1	600	466	167	88.1	46.8	72	31
150	21.7	600	425	293	80.3	17.4	138	97
200	0.7	NC*	--	--	--	--	--	--
300	0.7	NC	--	--	--	--	--	--
400	0.9	NC	--	--	--	--	--	--
500	1.0	NC	--	--	--	--	--	--
600	1.4	NC	--	--	--	--	--	--
700	1.4	NC	--	--	--	--	--	--
800	1.4	NC	--	--	--	--	--	--
900	1.4	NC	--	--	--	--	--	--
1000	1.5	NC	--	--	--	--	--	--

*Not cloned.

Table 128

MOUSE LYMPHOMA FORWARD MUTATION CONCENTRATION-RANGE ASSAY OF CROTOXYPHOS IN THE PRESENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency ($\times 10^{-6}$)	Induced Mutation Frequency ($\times 10^{-6}$)
Solvent control (1% DMSO)	99.7	600	590	300	106.5	106.2	102	--
	100.3	600	518	263	93.5	93.8	102	--
Positive control (5 μg/ml 3-MCA)	59.7	600	296	599	53.4	31.9	405	303
	60.3	600	365	548	65.9	39.7	300	198
Crotoxyphos								
1	108.2	600	584	205	105.4	114.1	70	--
2	108.2	600	482	192	87.0	94.1	80	--
5	92.9	600	482	267	87.0	80.8	111	9
10	92.9	600	454	352	81.9	76.1	155	53
20	81.4	600	475	383	85.7	69.8	161	59
40	43.5	600	472	532	85.2	37.1	225	123
80	13.5	600	286	506	51.6	7.0	354	252
100	5.3	600	148	376	26.7	1.4	508	406
150	1.1	NC*	--	--	--	--	--	--
200	1.3	NC	--	--	--	--	--	--
300	1.9	NC	--	--	--	--	--	--
400	2.3	NC	--	--	--	--	--	--
500	1.0	NC	--	--	--	--	--	--
600	1.4	NC	--	--	--	--	--	--
700	1.6	NC	--	--	--	--	--	--
800	0.3	NC	--	--	--	--	--	--
900	1.4	NC	--	--	--	--	--	--
1000	1.2	NC	--	--	--	--	--	--

---

*Not cloned.

Table 129

MOUSE LYMPHOMA FORWARD MUTATION ASSAY OF CROTOXYPHOS IN THE ABSENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency (× 10^{-6})	Induced Mutation Frequency (× 10^{-6})
Solvent control (1% DMSO)	95.8	600	601	151	104.8	100.4	50	--
	104.2	600	546	126	95.2	99.2	46	--
Positive control (500 μg/ml EMS)	68.1	600	401	1303	69.9	47.6	650	602
	68.2	600	519	1223	90.5	61.7	471	423
Crotoxyphos								
10	104.4	600	649	196	113.2	118.1	60	12
	99.2	600	512	119	89.3	88.6	46	--
30	87.1	600	621	184	108.3	94.3	59	11
	86.8	600	656	201	114.4	99.3	61	13
80	73.8	600	601	160	104.8	77.3	53	5
	73.8	600	624	251	108.8	80.3	80	32
100	63.8	600	478	259	83.3	53.2	108	60
	66.6	600	549	225	95.7	63.8	82	34
120	57.3	600	636	223	110.9	63.5	70	22
	56.2	600	530	227	92.4	51.9	86	38
150	39.2	600	510	267	88.9	34.9	105	57
	40.9	600	516	318	90.0	36.8	123	75
160	31.1	600	534	321	93.1	29.0	120	72
	35.0	600	517	428	90.1	31.6	166	118
170	22.0	600	557	279	97.1	21.4	100	52
	24.0	600	517	368	90.1	21.6	142	94
180	17.7	600	543	371	94.7	16.8	137	89
	14.8	600	544	406	94.9	14.0	149	101
200	1.7	NC*	--	--	--	--	--	--
	4.3	600	534	480	93.1	4.0	180	132

---

*Not cloned

Table 130

MOUSE LYMPHOMA FORWARD MUTATION ASSAY OF CROTOXYPHOS IN THE PRESENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency (× 10^{-6})	Induced Mutation Frequency (× 10^{-6})
Solvent control	98.3	600	508	152	101.9	100.2	60	--
(1% DMSO)	101.7	600	489	313	98.1	99.8	128*	--
Positive control	45.9	600	245	570	49.1	22.6	465	405
(5 μg/ml 3-MCA)	52.1	600	244	--†	48.9	25.5	--	--
Crotoxyphos								
5	101.7	600	548	540	109.9	111.8	197	137
	94.3	600	547	496	109.7	103.5	181	121
10	96.5	600	446	448	89.5	86.3	201	141
	79.3	600	531	577	106.5	84.5	217	157
20	80.7	600	507	615	101.7	82.1	243	183
	79.0	600	436	526	87.5	69.1	241	181
25	76.9	600	478	739	95.9	73.7	309	249
	71.5	600	544	775	109.2	78.0	285	225
30	56.8	600	495	666	99.3	56.4	269	209
	65.7	600	467	637	93.7	61.5	273	213
35	51.8	600	459	633	92.1	47.7	276	216
	44.6	600	428	598	85.9	38.3	279	219
40	41.9	600	484	503	97.1	40.7	208	148
	49.1	600	477	535	95.7	47.0	224	164
55	20.8	600	355	590	71.2	14.8	332	272
	21.3	600	310	897	62.2	13.2	579	519
70	13.2	600	262	804	52.6	6.9	614	554
	16.1	600	277	713	55.6	8.9	515	455
80	13.3	600	253	696	50.8	6.8	550	490
	12.5	600	271	671	54.6	6.8	495	435

---

*The spontaneous mutation frequency of one solvent control sample is not acceptable as it exceeds the assay standard of 100 × 10^{-6}. However, this assay was included in the evaluation of crotoxyphos because the spontaneous frequency of the other control was below 100 × 10^{-6} and the other criteria for a valid assay were met.

†Sample lost.

Table 131

DOSAGE AND SACRIFICE SCHEDULE

Compound	No. Animals	Dose at 0 and 24 Hr	Mean Body Weight Change (g)	No. Sacrificed			No. Fatalities
				30 Hr	48 Hr	72 Hr	
TMP--Positive control	12	1.25 g/kg	-1.6±.9	4	4	4	0
DMSO--Vehicle control	24	5 ml/kg	-0.7±.9	8	8	8	0
Crotoxyphos							
Low Dose	26	5 mg/kg	-0.8±.7	8	8	8	0
Medium Dose	26	10 mg/kg	-1.0±.8	8	8	8	1
High Dose	28	20 mg/kg	-0.8±1.1	6	5	8	8

Table 132

INCIDENCE OF MICRONUCLEATED POLYCHROMATOPHILIC ERYTHROCYTES IN BLOOD AND BONE MARROW*

(500 PCEs Scored per Smear)

	30 Hours				48 Hours				72 Hours			
Dose	Animal No.	Blood PCE with MN	BM PCE with MN	BM PCE/RBC	Animal No.	Blood PCE with MN	BM PCE with MN	BM PCE/RBC	Animal No.	Blood PCE with MN	BM PCE with MN	BM PCE/RBC
DMSO 5 ml/kg	C0	+	0	1.3	A0	1	0	0.7	B0	1	1	0.9
	C1	+	0	1.0	A1	0	0	1.3	B1	1	0	1.2
	C2	+	1	1.0	A2	0	1	0.9	B2	0	1	0.6
	C3	+	1	0.7	A3	0	0	1.4	B3	0	1	0.9
	D0	+	2	1.0	E0	0	0	0.9	F0	0	1	2.7
	D1	+	0	1.3	E1	1	0	1.0	F1	0	1	1.2
	D2	+	1	1.0	E2	0	0	0.9	F2	0	0	2.8
	D3	+	3	1.2	E3	1	0	0.7	F3	0	2	0.9
	Total	--	8			3	1			2	8	
	Mean			1.1±.1				1.0±.3				1.4±.9
TMP 1.25 g/kg	J0	2	30	0.6	H0	4	21	0.7	I0	6	8	0.5
	J1	3	10	0.3	H1	0	13	0.6	I1	2	0	0.8
	J2	6	8	0.6	H2	0	20	1.3	I2	3	3	0.3
	J3	+	20	0.4	H3	0	3	0.2	I3	1	0	0.7
	Total	11	68			4	57			12	11	
	Mean			0.5±.15				0.7±.5				0.6±.2
Crotoxyphos 5.0 mg/kg	GG0	1.5	0	1.0	FF0	0	0	1.0	KK0	0	2	2.4
	GG1	0	1	1.6	FF1	2	0	0.9	KK1	1	0	1.3
	GG2	2	0	1.2	FF2	0	1	1.1	KK2	0	0	1.2
	GG3	1.5	0	1.2	FF3	0	0	1.4	KK3	0	0	1.5
	JJ0	0	0	1.0	II0	1	0	1.2	HH0	1	0	0.9
	JJ1	2	3	0.8	II1	2	2	1.0	HH1	0	0	0.9
	JJ2	1.5	0	0.8	II2	0	0	0.8	HH2	1	0	1.1
	JJ3	2	0	0.7	II3	0	0	1.1	HH3	1	0	1.4
	Total	10.5	4			5	3			4	2	
	Mean			1.0±.3				1.1±.2				1.3±.5
Crotoxyphos 10 mg/kg	OO0	1	0	1.4	MM0	1	0	1.4	QQ0	0	0	0.2
	OO1	0	0	0.6	MM1	0	0	0.7	QQ1	0	0	1.4
	OO2	1	0	0.8	MM2	0	0	1.1	QQ3	0	0	1.4
	OO3	2	0	1.0	MM3	1	0	0.9	RR0	1	0	0.9
	PP0	1	0	0.6	NN0	2	1	1.6	RR1	0	1	1.2
	PP1	1	2	1.1	NN1	1	1	1.5	RR2	+	1	1.3
	PP2	2	1	0.8	NN2	1	0	1.2	RR3	+	0	1.3
	PP3	0	1	1.1	NN3	0	0	1.0	SS0	1	0	0.7
	Total	8	4			6	2			2	2	
	Mean			0.9±.3				1.2±.3				1.1±.4
Crotoxyphos 20 mg/kg	XX0	1	1	0.9	TT0	0	1	0.7	UU1	0	0	1.7
	XX2	0	1	1.1	TT1	1	0	1.5	UU2	1	0	1.1
	XX3	1	1	1.0	TT3	1	2	1.5	UU3	2	0	1.0
	ZZ0	1	1	0.9	WW0	1	0	0.7	YY1	0	0	1.1
	ZZ1	2	0	0.6	WW2	1	3	0.9	YY2	1	0	0.9
	ZZ3	1	0	1.2					YY3	0	0	1.6
									VV0	+	2	0.8
									VV2	+	1	1.0
	Total	6	4			4	6			4	3	
	Mean			1.0±.2				1.1±.4				1.1±.4

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*BM = bone marrow; PCE = polychromatophilic erythrocyte; RBC = mature erythrocyte; MN = micronucleus.
+Blood smears for these animals were not evaluated.

2,4D and 2,4DB Acids

S. typhimurium Plate Incorporation, E. coli WP-2, S. cerevisiae D3. Both of these compounds were previously found to be negative, with and without activation, in these three test systems (52).

E. coli/B. subtilis Relative Toxicity (Table 133). Both compounds were previously tested and reported to be positive in both species (52). These results were reexamined, and it was determined that 2,4DB should have been reported as negative in the *B. subtilis* test. At the EPA's request, 2,4D was retested under the present contract and the results indicated that it was negative in *E. coli*, rather than positive. In *B. subtilis*, the retest results for 2,4D were in agreement with the previous report.

S. typhimiurium Relative Toxicity (Tables 134-136). Both compounds were negative in both strain pairs.

UDS in WI-38 Cells. Both compounds were previously tested and found to be negative, both with and without activation (52).

Table 133

DIFFERENTIAL TOXICITY ASSAY

2,4-D ACID

Retest

Compound	Amount of Compound Added per Plate	Zone of Inhibition of Growth (in mm, disc = 6 mm)			
		E. coli		B. subtilis	
		W3110 ($polA^+$)	p3478 ($polA^-$)	H17 (rec^+)	M45 (rec^-)
Experiment 1					
Ampicillin	.01 mg	12	12	NT*	NT
Methylmethanesulfonate	1 mg	33	42	NT	NT
1-Phenyl-3,3-dimethyltriazene	1 mg	40	61	NT	NT
2,4-D acid	1 mg	10, 11, 11	11, 11, 11	NT	NT
	5 mg	13, 14, 13	14, 14, 14	NT	NT
Experiment 2					
Ampicillin	.01 mg	10	10	18	20
1-Phenyl-3,3-dimethyltriazene	1 mg	49	63	53	60
2,4-D acid	1 mg	8, 9	9, 9	10, 10	20, 20
	5 mg	11, 13, 11	13, 13, 10	17, 13, 16	24, 25, 25
Experiment 3					
Kanamycin	0.1 mg	11, 11	11, 11	15, 16	15, 16
Methylmethanesulfonate	1 mg	15, 14	25, 23	17, 17	50, 47
2-4-D acid	5 mg	10, 10	10, 10	21, 20	24, 24

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*NT, not tested.

Table 134

DIFFERENTIAL TOXICITY ASSAYS WITH SALMONELLA TYPHIMURIUM STRAINS

Experiment 1

Compound	Amount of Compound Applied to Disc	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		SL4525 (rec$^+$)		SL4700 (rec$^-$)		TA1978 (uvrB$^+$)		TA1538 (uvrB$^-$)	
Negative Control									
Kanamycin	100.0 μg	14	13	13	14	13	13	14	14
Positive Controls									
MMS*	10.0 mg	47	46	68	65				
4-NQO†	2.0 mg					12	12	24	23
2,4-D Acid	1.0 mg	24	24	20	19	16	17	16	15
	5.0 mg	28	28	24	24	20	18	17	18
2,4-DB Acid	1.0 mg	14	15	13	14	10	10	13	11
	5.0 mg	14	14	14	13	10	10	13	11

Table 135

DIFFERENTIAL TOXICITY ASSAYS WITH SALMONELLA TYPHIMURIUM STRAINS

Experiment 2

Compound	Amount of Compound Applied to Disc	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		SL4525 (rec$^+$)		SL4700 (rec$^-$)		TA1978 (uvrB$^+$)		TA1538 (uvrB$^-$)	
Negative Control									
Kanamycin	100.0 µg	15	14	16	15	17	17	15	16
Positive Controls									
MMS*	10.0 mg	45	40	72	74				
4-NQO†	2.0 mg					12	12	28	26
2,4-D Acid	1.0 mg	28	29	30	30	20	20	23	23
	5.0 mg	31	31	31	30	23	20	23	25
2,4-DB Acid	1.0 mg	18	18	20	20	10	10	13	14
	5.0 mg	19	17	21	21	10	10	16	16

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* MMS = Methylmethanesulfonate.

† 4-NOO = 4-Nitroquinoline-1-oxide.

Table 136

DIFFERENTIAL TOXICITY ASSAYS WITH SALMONELLA TYPHIMURIUM STRAINS

Experiment 3

		Zone of Inhibition of Growth (in mm, disc = 6 mm)							
Compound	Amount of Compound Applied to Disc	SL4525 (rec$^+$)		SL4700 (rec$^-$)		TA1978 (uvrB$^+$)		TA1538 (uvrB$^-$)	
Negative Control									
Kanamycin	100.0 µg	14	13	12	12	13	13	14	13
Positive Controls									
MMS[*]	10.0 mg	48	49	61	65				
4-NQO[†]	2.0 mg					20	21	28	27
2,4-D Acid	5.0 mg	13	13	13	11	14	13	12	14
	10.0 mg	19	21	22	23	20	23	21	21

---

[*] MMS = Methylmethanesulfonate.

[†] 4-NQO = 4-Nitroquinoline-1-oxide.

Demeton

S. typhimurium Plate Incorporation, E. coli WP-2, S. cerevisiae D3. Demeton was previously tested and was found to be positive, both with and without activation in all three systems (52).

S. cerevisiae D7 (Tables 137, 138). In the first experiment, demeton was tested over a concentration range of 0.01 to 0.1%. The second experiment tested concentrations over a narrower range--from 0.02 to 0.06%. In both assays, demeton elicited a positive response for mitotic crossing-over, gene conversion, and reverse mutation.

E. coli/B. subtilis Relative Toxicity. This compound was previously tested and reported to be positive in both species (52). Reexamination of these data indicates that demeton was negative in E. coli.

S. typhimurium Relative Toxicity (Tables 139-141). Demeton appeared to give unequal zones of growth inhibition, but the difference was marginal and we conclude that it is negative in both strain pairs.

SCE in CHO Cells (Tables 142-145). Table 142 presents the results of the cytotoxicity test of demeton in CHO cell cultures without metabolic activation. At concentrations of 0.1 and 0.5%, demeton was cytotoxic to CHO cells. At concentrations of 0.02% and less, M_2 metaphases were produced. Tables 143 presents the results of the cytotoxicity test of demeton in CHO cell cultures with metabolic activation. At concentrations of 0.1 and 0.5%, demeton was cytotoxic to CHO cells. At concentrations of 0.02% and less, primarily M_2 metaphases were produced.

Table 144 presents the SCE frequencies in CHO cells exposed to demeton without metabolic activation. Concentrations of 0.01 and 0.02% produced only M_1 metaphases and were not suitable for scoring. A one-way analysis of variance was conducted on SCE frequencies of the three lower concentrations of demeton and of the negative controls. The variance between treatment groups was significantly greater than the variance within treatment groups. The response appeared to be concentration-related. The maximum effect (approximately 3.9 to 4.8 times the negative control SCE frequencies) was seen at 0.005%.

Table 145 presents the SCE frequencies in CHO cells exposed to demeton with metabolic activation. One sample exposed to 0.02% demeton was lost during harvest. For the other four concentrations of demeton and the negative control, the variance between treatment groups was significantly greater than the variance within treatment groups. The response appeared to be dose-related, the maximum effect (approximately 2.9 to 3.6 times the negative control SCE frequencies) was seen at 0.04%.

The test of demeton with metabolic activation involved exposing CHO cells to a higher concentration range for a shorter time (0.0025 to 0.04% for 2 hours) than that used in the test without metabolic activation (0.00125 to 0.005% for 21.5 hours). Therefore, direct comparisons are inappropriate. However, we conclude that demeton increases the SCE frequencies in CHO cells, both with and without activation.

L5178Y Mouse Lymphoma (Tables 146-150). Table 146 presents the results of concentration-range testing of demeton in the absence of a metabolic activation system. The results showed that the range to use in mutagenesis assays was approximately 5 to 100 µg/ml (106.1% relative total growth at 5 µg/ml and 15.9% relative total growth at 100 µg/ml). Concentration-range test results for demeton in the presence of the metabolic activation system (Table 145) showed that the approximate range to use in the mutagenesis assays was 40 to 150 µg/ml (109.4% relative total growth at 40 µg/ml and 5.8% relative total growth at 150 µg/ml).

Tables 148 and 149 present the results of mutagenesis testing of demeton in the absence of the metabolic activation system. At the high concentration (100 µg/ml) in both assays, the average relative total growth was 12.7% and 11.9%. At the low concentration (5 µg/ml) the average relative total growth was 75.7% and 104.8%. From 5 to 100 µg/ml, a concentration-related increase in induced mutation frequency was observed in both assays. Mutation frequencies at least twice the spontaneous frequency were observed from 37 to 100 µg/ml (the seven highest concentrations) in the repeat assay.

Table 150 presents the results of mutagenesis testing of demeton in the presence of the S-9 metabolic activation system. Demeton was most cytotoxic at concentrations above 150 µg/ml, with relative total growth values of less than 10.0%. No significant depression in the relative total growth values was observed at or below 50 µg/ml, which yielded an average relative total growth of 98.8%. In the concentration range of 20 to 150 µg/ml (eight concentrations), there were no concentrations that yielded a mutation frequency at least twice the spontaneous frequency with relative total growth values of at least 10.0%.

These combined results indicate a positive mutagenic effect of demeton at the thymidine kinase locus in L5178Y mouse lymphoma cells only in the absence of the metabolic activation system. In the absence of the metabolic activation system demeton increased the mutation frequency, in a concentration-related manner, above the spontaneous frequency of the control. The presence of the metabolic activation system appeared to decrease both the cytotoxicity and mutagenic responses of L5178Y $TK^{+/-}$ cells to demeton.

UDS in WI-38 Cells. Demeton was previously tested and found to be positive, both with and without activation (52).

Micronucleus Test (Tables 151,152). The dosing and sacrifice schedules are shown in Table 151. The incidences of micronuclei in PCEs and the PCE to RBC ratios are given in Table 152. The vehicle control and the demeton-treated groups were negative at all dose levels and sampling times ($\beta < 0.05$). The reasons for the elevated PCE to RBC ratios in two DMSO animals and three low-dose demeton animals at 96 hours is not known. Ratios for all other dose/time groups were within normal limits ($\bar{x} = 0.50$ to 1.50).

The incidence of some fatalities, together with the mean weight loss in all dosed groups, indicates that a maximum tolerated dose was administered. The low incidences of micronuclei at all dose/time points indicate that demeton is not mutagenic in the micronucleus test according to the criteria used.

Table 137

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D7

DEMETON

Experiment 1

Compound	MA	% Conc.	Survivors: Cells Per ml ($\times 10^{-7}$)	Survivors: %	Crossing-Over: Mitotic Recombinants: Per ml ($\times 10^{-3}$)	Crossing-Over: Mitotic Recombinants: Per 10^5 Survivors	Crossing-Over: Total Aberrants: Per ml ($\times 10^{-3}$)	Crossing-Over: Total Aberrants: Per 10^5 Survivors	Gene Conversion: Convertants: Per ml ($\times 10^{-1}$)	Gene Conversion: Convertants: Per 10^6 Survivors	Reverse Mutation: Revertants: Per ml ($\times 10^0$)	Reverse Mutation: Revertants: Per 10^7 Survivors
Negative Control												
DMSO	-	10	5.2	100	4.5	8.7	6.5	13	70	13	14	2.7
	+	10	5.6	100	1.5	2.7	11	20	66	12	16	2.9
Positive Control												
1,2,3,4-Diepoxybutane	-	0.013	3.9	75	96	250	250	640	1900	490	280	72
	+	0.013	3.7	66	92	250	370	1000	1600	430	330	89
Demeton	-	0.01	4.0	77	4.0	10	21	53	79	20	18	4.5
	-	0.025	2.9	56	0	0	15	52	200	69	13	4.5
	-	0.05	3.2	62	8.0	25	54	170	240	75	73	23
	-	0.075	0.7	13	11	160	45	640	240	340	53	76
	-	0.1	1.5	29	29	190	95	630	350	230	14	9.3
	+	0.01	3.8	68	1.0	2.6	20	53	84	22	13	3.4
	+	0.025	4.9	88	16	33	25	51	100	20	34	6.9
	+	0.05	2.4	43	14	58	42	180	230	96	86	36
	+	0.075	0.65	12	16	250	79	1200	420	650	30	46
	+	0.1	0.83	15	37	450	130	1600	460	550	22	27

Table 138

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D7

DEMETON

Experiment 2

Compound	MA	% Conc.	Survivors: Cells Per ml ($\times 10^{-7}$)	Survivors: %	Crossing-Over: Mitotic Recombinants: Per ml ($\times 10^{-3}$)	Crossing-Over: Mitotic Recombinants: Per 10^5 Survivors	Crossing-Over: Total Aberrants: Per ml ($\times 10^{-3}$)	Crossing-Over: Total Aberrants: Per 10^5 Survivors	Gene Conversion: Convertants: Per ml ($\times 10^{-1}$)	Gene Conversion: Convertants: Per 10^6 Survivors	Reverse Mutation: Revertants: Per ml ($\times 10^0$)	Reverse Mutation: Revertants: Per 10^7 Survivors
Negative Control												
DMSO	-	10	3.5	100	1.0	2.9	8.0	23	61	17	20	5.7
	+	10	3.5	100	1.0	2.9	11	31	54	15	22	6.3
Positive Control												
1,2,3,4-Diepoxybutane	-	0.013	1.5	43	67	450	340	2300	1000	670	450	300
	+	0.013	2.3	66	44	190	370	1600	1200	520	410	180
Demeton	-	0.02	4.8	137	4.0	8.3	48	100	320	67	85	18
	-	0.03	2.7	77	3.0	11	45	170	260	96	36	13
	-	0.04	1.2	34	17	140	81	680	110	92	21	18
	-	0.05	0.58	17	7.0	120	52	900	59	100	5.0	8.6
	-	0.06	0.27	7.0	13	480	34	1300	43	160	7.0	26
	+	0.02	5.4	154	2.0	3.7	31	57	220	41	63	12
	+	0.03	6.1	174	5.0	8.2	61	100	320	52	100	16
	+	0.04	4.8	137	7.0	15	60	130	330	69	82	17
	+	0.05	2.0	57	15	75	45	230	140	70	29	15
	+	0.06	0.33	4.9	11	330	44	1300	56	170	11	33

Table 139

DIFFERENTIAL TOXICITY ASSAYS WITH SALMONELLA TYPHIMURIUM STRAINS

Experiment 1

Compound	Amount of Compound Applied to Disc	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		SL4525 (rec$^+$)		SL4700 (rec$^-$)		TA1978 (uvrB$^+$)		TA1538 (uvrB$^-$)	
Negative Control									
Kanamycin	**100 μg**	**15**	**16**	**15**	**15**	**14**	**15**	**15**	**16**
Positive Controls									
MMS*	**10 mg**	**53**	**53**	**70**	**70**				
4-NQO†	**2 mg**					**12**	**12**	**24**	**25**
Demeton	5 μl	11	12	10	9	7	9	7	8
	10 μl	13	16	16	18	9	8	9	9

---

*MMS = Methylmethanesulfonate.

†4-NQO = 4-Nitroquinoline-1-oxide.

Table 140

DIFFERENTIAL TOXICITY ASSAYS WITH SALMONELLA TYPHIMURIUM STRAINS

Experiment 2

Compound	Amount of Compound Applied to Disc	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		SL4525 (rec^+)		SL4700 (rec^-)		TA1978 ($uvrB^+$)		TA1538 ($uvrB^-$)	
Negative Control									
Kanamycin	100.0 µg	15	14	16	15	17	17	15	16
Positive Controls									
MMS*	10.0 mg	45	40	72	74				
4-NQO†	2.0 mg					12	12	28	26
Demeton	10.0 µl	13	12	12	10	10	9	9	9
	20.0 µl	13	13	15	15	11	11	14	15

---

* MMS = Methylmethanesulfonate.

† 4-NOO = 4-Nitroquinoline-1-oxide.

Table 141

DIFFERENTIAL TOXICITY ASSAYS WITH SALMONELLA TYPHIMURIUM STRAINS

Experiment 3

Compound	Amount of Compound Applied to Disc	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		SL4525 (rec^+)		SL4700 (rec^-)		TA1978 ($uvrB^+$)		TA1538 ($uvrB^-$)	
Negative Control									
Kanamycin	100.0 µg	14	13	12	12	13	13	14	13
Positive Controls									
MMS*	10.0 mg	48	49	61	65				
4-NQO†	2.0 mg					20	21	28	27
Demeton	20.0 µl	11	12	14	14	12	14	16	16
	30.0 µl	15	17	16	16	14	16	19	17

---

*MMS = Methylmethanesulfonate.

†4-NQO = 4-Nitroquinoline-1-oxide.

Table 142

CYTOTOXICITY TEST OF CHINESE HAMSTER OVARY CELLS EXPOSED TO DEMETON FOR 21.5 HOURS WITHOUT METABOLIC ACTIVATION

Treatment	Number of Divisions in Bromodeoxyuridine (BrdU)[*]
Negative control (1% DMSO)	$M_2 > M_1$[†]
Demeton (%)	
0.0008	$M_2 > M_1$[†]
0.004	$M_2 > M_1$[†]
0.02	$M_2 > M_1$[†]
0.1	--[‡]
0.5	--[‡]
Positive control (10^{-3} M ethyl methanesulfonate)	$M_2 > M_1$[†]

---

[*] M_1, one division in BrdU; M_2, two divisions in BrdU.

[†] The type of cell to the left of the > sign was in excess.

[‡] Demeton was cytotoxic to CHO cultures at this concentration.

Table 143

CYTOTOXICITY TEST OF CHINESE HAMSTER OVARY CELLS EXPOSED TO DEMETON FOR 2 HOURS WITH METABOLIC ACTIVATION

Treatment	Number of Divisions in Bromodeoxyuridine (BrdU)[*]
Negative control (1% DMSO)	$M_2 >> M_1$[†]
Demeton (%)	
0.0008	$M_2 >> M_1$[†]
0.004	$M_2 >> M_1$[†]
0.02	$M_2 > M_1$[‡]
0.1	--[§]
0.5	--[§]
Positive control (10^{-3} M dimethylnitrosamine)	$M_2 >> M_1$[†]

---

[*] M_1, one division in BrdU; M_2, two divisions in BrdU.

[†] The cell type to the left of >> sign was in great excess.

[‡] The cell type to the left of the > sign was in excess.

[§] Demeton was cytotoxic at this concentration.

Table 144

SISTER CHROMATID EXCHANGE (SCE) FREQUENCIES IN CHINESE HAMSTER OVARY (CHO) CELLS EXPOSED TO DEMETON FOR 21.5 HOURS WITHOUT METABOLIC ACTIVATION

Treatment	Scorer*	No. of SCEs	No. of Chromosomes	Chromosomes/Cell (Mean ± SD)	SCEs/Cell† (Mean ± SE)	SCEs/ Chromosome† (Mean ± SD)
Negative control (1% DMSO)	A	318	486	19.44 ± 0.58	12.72 ± 0.71	0.654 ± 0.037
	B	286	502	20.08 ± 0.76	11.44 ± 0.68	0.570 ± 0.034
Demeton (%)						
0.00125	A	625	477	19.08 ± 1.00	25.00 ± 1.00	1.310 ± 0.052
	B	658	499	19.96 ± 0.75	26.32 ± 1.03	1.319 ± 0.051
0.0025	A	950	480	19.20 ± 0.71	38.00 ± 1.23	1.979 ± 0.064
	B	1064	501	20.04 ± 0.61	42.56 ± 1.31	2.124 ± 0.065
0.005	A	1226	482	19.28 ± 0.74	49.04 ± 1.40	2.544 ± 0.073
	B	1354	494	19.76 ± 1.01	54.16 ± 1.47	2.741 ± 0.074
0.01		--‡	--	--	--	--
0.02		--‡	--	--	--	--
Positive control (10^{-3} M ethyl methanesulfonate)	A	1054	485	19.40 ± 0.76	42.16 ± 1.30	2.173 ± 0.067
	B	928	497	19.88 ± 0.93	39.28 ± 1.24	1.867 ± 0.063

---

[*] Each scorer analyzed 25 cells per sample.

[†] A one-way analysis of variance comparing the SCE frequencies in CHO cells exposed to demeton and to the negative control indicated that the variance between treatment groups was significantly greater ($P < 0.0005$) than the variance within treatment groups.

[‡] Only M_1 metaphases produced.

Table 145

SISTER CHROMATID EXCHANGE (SCE) FREQUENCIES IN CHINESE HAMSTER OVARY (CHO) CELLS
EXPOSED TO DEMETON FOR 2 HOURS WITH METABLOIC ACTIVATION

Treatment	Scorer*	No. of SCEs	No. of Chromosomes	Chromosomes/Cell (Mean ± SE)	SCEs/Cell† (Mean ± SE)	SCEs/ Chromosome† (Mean ± SD)
Negative control	A	247	456	18.24 ± 0.83	9.88 ± 0.63	0.542 ± 0.034
(1% DMSO)	B	326	483	19.32 ± 0.63	13.04 ± 0.72	0.675 ± 0.037
Demeton (%)						
0.0025	A	283	456	18.24 ± 1.05	11.32 ± 0.67	0.621 ± 0.037
	B	337	491	19.64 ± 0.81	13.48 ± 0.73	0.686 ± 0.037
0.005	A	317	477	19.08 ± 0.70	12.68 ± 0.71	0.665 ± 0.037
	B	338	477	19.08 ± 0.70	13.52 ± 0.74	0.709 ± 0.039
0.01	A	348	462	18.48 ± 0.87	13.92 ± 0.75	0.753 ± 0.040
	B	424	479	19.16 ± 0.80	16.96 ± 0.82	0.885 ± 0.043
0.02		--‡	--	--	--	--
0.04	A	898	465	18.60 ± 1.12	35.92 ± 1.20	1.931 ± 0.064
	B	939	479	19.16 ± 0.80	37.56 ± 1.23	1.960 ± 0.064
Positive control	A	839	471	18.84 ± 1.03	33.56 ± 1.16	1.781 ± 0.061
(10^{-3} M dimethylnitrosamine)	B	1014	478	19.16 ± 0.97	40.56 ± 1.27	2.157 ± 0.067

---

[*] Each scorer analyzed 25 cells per sample.

[†] A one-way analysis of variance comparing the SCE frequencies in CHO cells exposed to demeton and to the negative control indicated that the variance between treatment groups was significantly greater ($P < 0.0005$) than the variance within treatment groups.

[‡] One sample was lost during harvest.

Table 146

MOUSE LYMPHOMA FORWARD MUTATION CONCENTRATION-RANGE ASSAY OF DEMETON IN THE ABSENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency ($\times 10^{-6}$)	Induced Mutation Frequency ($\times 10^{-6}$)
Solvent control (1% DMSO)	80.8	600	559	156	106.8	86.3	56	--
	119.3	600	488	130	93.2	111.2	53	--
Positive control (500 μg/ml EMS)	78.5	600	366	959	69.9	54.9	524	470
	72.8	600	320	1002	61.1	44.5	626	571
Demeton								
1	109.4	600	457	180	87.3	95.5	79	24
2	113.8	600	480	168	91.7	104.3	70	15
5	104.0	600	534	183	102.0	106.1	69	14
10	108.8	600	492	153	94.0	102.3	62	7
20	104.0	600	496	276	94.7	98.5	111	56
40	88.4	600	452	330	86.3	76.3	146	91
80	43.4	600	327	463	62.5	27.1	283	228
100	24.9	600	335	526	64.0	15.9	314	259
150	11.4	600	112	430	21.4	2.4	768	713
200	5.0	600	--*	324	--	--	--	--
300	1.1	NC†	--	--	--	--	--	--
400	0.4	NC	--	--	--	--	--	--
500	0.5	NC	--	--	--	--	--	--
600	0.4	NC	--	--	--	--	--	--
700	0.4	NC	--	--	--	--	--	--
800	0.6	NC	--	--	--	--	--	--
900	1.3	NC	--	--	--	--	--	--
1000	1.1	NC	--	--	--	--	--	--

---

*Sample lost.

†Not cloned.

Table 147

MOUSE LYMPHOMA FORWARD MUTATION CONCENTRATION-RANGE ASSAY OF FEMETON IN THE PRESENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency ($\times 10^{-6}$)	Induced Mutation Frequency ($\times 10^{-6}$)
Solvent control	98.5	600	538	223	100.9	99.4	83	--
(1% DMSO)	101.5	600	528	233	99.1	100.5	88	--
Positive control	61.6	600	367	623	68.9	42.4	340	254
(5 μg/ml 3-MCA)	68.0	600	396	617	74.3	50.5	312	226
Demeton								
1	106.4	600	586	262	109.9	116.9	89	3
2	108.0	600	522	268	97.9	105.8	103	17
5	99.9	600	547	263	102.6	102.5	96	10
10	104.5	600	551	252	103.4	108.0	91	5
20	106.4	600	518	247	97.2	103.4	95	9
40	113.2	600	515	260	96.6	109.4	101	15
80	78.0	600	478	209	89.7	70.0	87	1
100	34.0	600	577	287	108.3	36.8	99	13
150	6.4	600	486	356	91.2	5.8	147	61
200	7.2	600	352	383	66.0	4.8	218	132
300	3.9	NC*	--	--	--	--	--	--
400	1.6	NC	--	--	--	--	--	--
500	1.7	NC	--	--	--	--	--	--
600	0.5	NC	--	--	--	--	--	--
700	0.6	NC	--	--	--	--	--	--
800	0.4	NC	--	--	--	--	--	--
900	0.4	NC	--	--	--	--	--	--
1000	0.4	NC	--	--	--	--	--	--

---

*Not cloned.

Table 148

MOUSE LYMPHOMA FORWARD MUTATION ASSAY OF DEMETON IN THE ABSENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (µg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency ($\times 10^{-6}$)	Induced Mutation Frequency ($\times 10^{-6}$)
Solvent control (1% DMSO)	106.9	600	532	147	99.5	106.4	55	--
	93.1	600	537	147	100.5	93.5	55	--
Positive control (500 µg/ml EMS)	66.3	600	326	945	61.0	40.4	580	525
	71.5	600	384	981	71.8	51.4	511	456
Demeton								
5	94.2	600	459	211	85.9	80.9	92	37
	90.1	600	418	162	78.2	70.5	78	23
10	86.3	600	529	264	99.0	85.4	100	45
	94.2	600	466	228	87.2	82.1	98	43
20	96.4	600	487	333	91.1	87.8	137	82
	93.0	600	511	235	95.6	88.9	92	37
37	77.8	600	420	316	78.6	61.1	150	95
	82.2	600	463	389	86.6	71.2	168	113
50	57.4	600	442	459	82.7	47.5	208	153
	78.8	600	398	424	74.5	58.7	213	158
60	53.0	600	419	510	78.4	41.5	243	188
	53.0	600	464	483	86.8	46.0	208	153
70	43.5	600	417	534	78.0	33.9	256	201
	43.5	600	481	522	90.0	39.1	217	162
80	39.1	600	427	550	79.9	31.2	258	203
	33.1	600	403	529	75.4	25.0	263	208
90	23.4	600	372	557	69.6	16.3	299	244
	21.5	600	357	537	66.8	14.4	301	246
100	20.7	600	325	522	60.8	12.6	321	266
	20.1	600	344	584	64.4	12.9	340	285

Table 149

REPEAT MOUSE LYMPHOMA FORWARD MUTATION ASSAY OF DEMETON IN THE ABSENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (µg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency (× 10⁻⁶)	Induced Mutation Frequency (× 10⁻⁶)
Solvent control (1% DMSO)	98.3	600	588	[illegible]	108.1	106.3	38	--
	101.7	600	500	125	91.9	93.5	50	--
Positive control (500 µg/ml EMS)	79.2	600	385	912	70.8	56.1	474	430
	68.4	600	384	794	70.6	48.3	414	370
Demeton								
1	113.0	NC*	--	--	--	--	--	--
	102.4	NC	--	--	--	--	--	--
5	102.4	600	580	178	106.6	109.2	61	17
	106.5	600	513	168	94.3	100.4	65	21
10	97.6	600	513	--†	94.3	92.0	--	--
	98.3	600	554	--†	101.8	100.1	--	--
20	93.6	600	460	357	84.6	79.1	155	111
	93.6	600	459	255	84.4	79.0	111	67
37	82.3	600	452	--†	84.4	69.4	--	--
	79.2	600	397	398	73.0	57.8	201	157
50	70.8	600	412	411	75.7	53.6	200	156
	68.2	600	441	402	81.1	55.3	182	138
60	56.6	600	359	443	66.0	37.4	247	203
	55.8	600	425	473	78.1	43.6	223	179
70	41.9	600	393	540	72.2	30.3	275	231
	43.1	600	375	490	68.9	29.7	261	217
85	30.9	600	362	466	66.5	20.6	257	213
	31.6	600	315	536	57.9	18.3	340	296
100	23.3	600	260	527	47.8	11.1	405	361
	23.5	600	296	510	54.4	12.8	345	301

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[*] Not cloned.

[†] Sample lost.

Table 150

MOUSE LYMPHOMA FORWARD MUTATION ASSAY OF DEMETON IN THE PRESENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency ($\times 10^{-6}$)	Induced Mutation Frequency ($\times 10^{-6}$)
Solvent control (1% DMSO)	105.2	600	493	194	99.6	104.8	79	--
	94.8	600	497	202	100.4	95.2	81	--
Positive control (7 μg/ml 3-MCA)	44.1	600	216	485	43.6	19.2	449	369
	48.1	600	250	466	50.5	24.3	373	293
Demeton								
20	97.2	600	520	257	105.1	102.1	99	19
	105.1	600	496	231	100.2	105.3	93	13
50	99.7	600	478	164	96.6	96.3	69	--
	97.4	600	515	203	104.0	101.3	79	--
70	71.1	600	519	189	104.8	74.5	73	--
	71.3	600	588	205	118.8	84.7	52	--
80	61.8	600	677	154	136.8	84.5	46	--
	71.1	600	597	183	120.6	85.8	61	--
100	55.5	600	474	210	95.8	53.1	89	9
	50.1	600	530	183	107.1	53.6	69	--
120	39.5	600	497	250	100.4	39.7	101	21
	36.9	600	474	207	95.8	35.3	87	7
130	25.9	600	462	220	93.3	24.2	95	15
	29.2	600	529	187	106.9	31.2	71	--
150	24.1	600	332	242	67.1	16.2	146	66
	23.5	600	423	234	85.5	20.1	111	31
200	13.7	600	330	272	66.7	9.1	165	85
	14.9	600	302	274	61.0	9.1	181	101
300	9.4	600	296	365	59.8	5.6	247	167
	9.6	600	354	366	71.5	6.9	207	127

Table 151

DOSING AND SACRIFICE SCHEDULE

Compound	No. Animals	Dose at		Mean Body Wt Change (g)	Number Sacrificed			No. Fatalities
		0 Hr	24 Hr		48 Hr	72 Hr	96 Hr	
DMSO	24	5 ml/kg	5 ml/kg	-1.6	7	7	7	3
Demeton								
Low dose	24	0.8 mg/kg	0.8 mg/kg	-1.1	8	8	7	1
Medium dose	24	1.6 mg/kg	1.6 mg/kg	-2.1	8	8	6	2
High dose	24	3.2 mg/kg	3.2 mg/kg	-1.2	8	7	8	1

Table 152

INCIDENCE OF MICRONUCLEATED POLYCHROMATOPHILIC ERYTHROCYTES*
(500 PCEs Scored per Animal)

Dose	48 Hours			72 Hours			96 Hours		
	Animal No.	PCEs with MN	PCE/RBC	Animal No.	PCEs with MN	PCE/RBC	Animal No.	PCEs with MN	PCE/RBC
DMSO 2 x 5 ml/kg (vehicle control)	47ø	0	0.72	48ø	0	0.82	46ø	3	1.10
	47r	0	0.71	48r	1	0.64	46r	1	2.47
	47ℓ	0	0.83	48ℓ	2	0.67	46ℓ	4	1.00
	47b	1	1.23	51ø	1	0.67	46b	1	1.23
	50r	1	0.47	51r	2	0.94	49ø	0	1.52
	50ℓ	0	0.86	51ℓ	3	1.04	49r	1	1.24
	50b	0	0.27	51b	0	1.02	49ℓ	1	4.00
	Total	2			9			11	
	Average		0.73			0.83			1.79
Demeton 2 x 0.8 mg/kg	30ø	3	0.54	27ø	0	0.89	28ø	0	1.20
	30r	0	0.52	27r	0	1.37	28r	1	2.99
	30ℓ	0	0.74	27ℓ	1	1.03	28ℓ	3	5.00
	30b	1	0.85	27b	0	0.64	29ø	1	2.38
	31ø	1	0.94	32ø	1	1.27	29r	2	1.19
	31r	1	0.98	32r	1	1.96	29ℓ	0	1.57
	31ℓ	1	0.83	32ℓ	2	0.97	29b	0	1.23
	31b	2	0.92	32b	1	0.88			
	Total	9			6			7	
	Average		0.79			1.13			2.22
Demeton 2 x 1.6 mg/kg	33ø	2	0.86	34ø	2	0.84	35ø	0	1.01
	33r	2	1.18	34r	0	1.00	35r	0	1.67
	33ℓ	1	1.00	34ℓ	1	1.01	36ø	0	2.10
	33b	0	1.23	34b	2	1.10	36r	1	1.46
	38ø	3	1.43	37ø	1	1.45	36ℓ	0	1.47
	38r	1	0.78	37ℓ	2	0.80	36b	1	1.10
	38ℓ	1	0.88	37r	0	0.49			
	38b	1	0.97	37b	0	0.55			
	Total	11			8			2	
	Average		1.04			0.91			1.47
Demeton 2 x 3.2 mg/kg	40ø	1	0.86	41ø	0	1.21	39ø	1	1.27
	40r	0	1.00	41r	1	1.32	39r	0	0.60
	40ℓ	0	1.18	41b	1	0.85	39ℓ	0	1.27
	40b	1	1.13	44ø	0	1.39	39b	1	0.61
	43ø	1	1.56	44r	0	0.70	42ø	0	1.63
	43r	1	0.77	44ℓ	2	0.97	42r	1	1.79
	43ℓ	1	1.21	44b	0	1.02	42ℓ	0	0.90
	43b	1	0.97				42b	0	1.15
	Total	6			4			3	
	Average		1.09			1.07			1.15

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*PCE = polychromatophilic erythrocyte
RBC = mature erythrocyte
MN = micronuclei

Diallate

S. typhimurium Plate Incorporation (Tables 153, 154). Diallate was previously tested and found to be positive only with activation (54). Under the present contract, it was tested twice over a dose range of 0.0005 to 0.1 µg/plate on separate days, using two plates per dose. A reproducible, dose-related increase in the number of histidine revertants was obtained with strains TA1535, TA98, and TA100 only in the presence of metabolic activation. Strains TA1535 and TA100, which are capable of detecting base-pair substitution mutagens, exhibited a stronger response than did strain TA98. Diallate did not appear mutagenic in strains TA1537 and TA1538.

E. coli WP-2. Diallate was previously tested and found negative with and without activation in this system (54).

S. cerevisiae D3. This compound was previously tested and found to be positive only with activation in this test system (54).

S. cerevisiae D7 (Tables 155-157). No genetic damage was observed in this assay for the detection of crossing-over, gene conversion, and reverse mutation. Diallate was evaluated in the D7 assay over concentrations ranging from 0.001 to 0.025%, 0.00075 to 0.0075%, and 0.0005 to 0.003%. Toxicity was observed at 0.025% without metabolic activation. The increases in the frequency of crossing-over and gene conversion in Experiment 1 appear to be due to a decrease in survival. This should not be interpreted as a positive response because there is no increase in the number of genetically altered colonies per milliliter, which is one of the criteria of a positive response. Thus, diallate was negative in this test system.

L5178Y Mouse Lymphoma (Tables 158-163). Table 158 presents the results of concentration-range testing of diallate in the absence of metabolic activation. The results showed that the range to use in mutagenesis assays was approximately 10.0 µg/ml to 60.0 µg/ml. This determination was based on 93.3% relative total growth at 10.0 µg/ml and 13.2% relative growth at 60.0 µg/ml. Concentration-range test results of diallate in the presence of metabolic activation (Table 159) showed that the approximate range to use in the mutagenesis assays was 2.0 µg/ml to somewhat more than 10.0 µg/ml, based on 100.4% relative total growth at 2.0 µg/ml and 24.7% at 10.0 µg/ml.

Tables 160 and 161 present the results of mutagenesis testing of diallate in the absence of metabolic activation. This compound was extremely toxic at concentrations greater than 60.0 µg/ml in both assays, yielding average relative total growth values of less than 13.3 and 9.7%. No significant depression in the relative total growth value was observed at or below 4.0 and 12.0 µg/ml in the two assays; these concentrations yielded average relative growth values of 98.2 and 99.4%. From approximately 4.0 to 60.0 µg/ml, a concentration-related increase in induced mutation was observed in both assays. A mutation frequency at least twice the spontaneous frequency was not observed for samples in one assay, but in the other assay a mutation frequency at least twice the spontaneous frequency was observed at the two highest concentrations with average relative total growth values of at least 9.6%. These results indicate a weak positive mutagenic response.

Tables 162 and 163 present the results of mutagenesis testing of diallate in the presence of metabolic activation. At the highest concentrations of 20.0 and 25.0 μg/ml in the two assays, the average relative total growth values were 16.9 and 7.3%. No significant depression in relative total growth was observed at or below 7.0 μg in one assay, which yielded an average relative total growth value of 103.4%. In the other assay, the concentration of 7.0 μg/ml yielded an average relative total growth value of 64.3%. From approximately 7.0 to 25.0 μg/ml, a concentration-related increase in the induced mutation frequency was observed. An average mutation frequency at least twice the spontaneous frequency was observed at the three highest concentrations, in both assays, with average relative total growth values of over 10.0%. These results indicate a positive mutagenic response.

These combined results provide evidence that diallate increased the mutation frequencies, in a concentration-related manner, above the spontaneous control frequencies. The presence of the metabolic activation system was not required to elicit the positive mutagenic response; but it did appear to enhance the cytotoxicity and mutagenicity of diallate.

Drosophila SLRL (Tables 164-166). Table 164 presents the effects of diallate on the viability and sterility of $y\ B/y^{+}Y$ males. The experimental design of the wide-range pretest called for eight 1:5 dilutions of diallate. Dilution errors caused a slight deviation from this plan. Green guts were observed in males exposed to each tested concentration of diallate, which indicated that palatability was not a problem. The wide-range pretest demonstrated that flies were killed by exposure to diallate at concentrations of $\geq$ 0.01% for 3 days. A concentration of approximately 0.002% diallate appeared to represent an LD$_{50}$. Fertility in surviving males was good. The narrow-range pretest was performed to select the most appropriate dose more precisely. In this experiment, concentrations of $\geq$ 0.001% diallate killed all the flies. At a concentration of 0.0005% diallate, 40% survived, and 75% of these males were fertile. At a concentration of 0.00025% diallate, 90% of the males survived and all were fertile. Therefore, 0.0004% diallate was chosen as the concentration expected to approximate an LD50.

Table 165 summarizes the results of the testing of diallate for SLRL mutations. Diallate was tested three times with concurrent positive and negative controls to achieve a sample size that was adequate to detect a 0.2% increase in the mutation frequency. A total of 8816 X-chromosomes from the gametes of 244 diallate-fed *Drosophila melanogaster* males were sampled for mutagenicity in this study. In the solvent control group, 8807 chromosomes from 211 male flies were tested. In the positive control (0.01% EMS) group, 416 chromosomes from the 27 treated males were assessed.

Table 166 presents the SLRL mutation frequencies observed in $y\ B/y^{+}Y$ males fed 0.0004% diallate for 3 days and in concurrently tested positive and solvent controls. Cluster lethals consisting of more than three mutations derived from one treated male in the diallate or solvent control group were not included in the computation because of their probable origins as spermatogonial events. The overall SLRL mutation frequencies obtained were 0.27% for the solvent control group, 0.47% for diallate, and 18.5% for the positive control group. The difference in mutation frequencies between the diallate and solvent control groups was 0.2%, the minimum difference recommended by the Drosophila Gene-Tox Committee* for establishing a significantly increased genetic risk associated with a chemical mutagen. When these results were analyzed using the Kastenbaum-Bowman test, the increased mutation frequency observed in the diallate-fed Drosophila was significant at the 5% level.

This study was not designed to sample separate broods (representing chemical exposure at various stages during spermatogenesis) in sufficient numbers to establish the statistical significance of any observed differences. Rather, a brooding scheme was selected to provide preliminary evidence for or against a marked stage-specific sensitivity to a test chemical. Positive results might suggest the need for further study.

Broods A, B, and C represent, respectively, gametes that were primarily mature sperm, spermatids, and spermatocytes undergoing meiosis at the termination of exposure to the chemical (47). In Brood C, the elevation in the overall mutation frequency observed in the diallate-fed flies compared to that of the solvent controls is quite striking (0.81% vs. 0.47%). Brood C mutation rates in the diallate group remained reasonably constant in Experiments I and III (no results in Experiment II); the extremely high Brood C mutation rate for the solvent controls in Experiment III is presumed to be a spurious effect.

If confirmed, this heightened sensitivity of the peri-meiotic brood to diallate would be quite interesting. The greatest sensitivity to ultimate carcinogens is generally observed in mature sperm (Brood A), whereas spermatids (Brood B) are usually most sensitive to procarcinogens (55). 1,2-Dibromoethane and 1,2-dibromopropane have also been reported to elevate the mutation frequency in the meiotic brood, but in that case spermatids were also affected (56).

In conclusion, the herbicide diallate fed to Drosophila males at a concentration of 0.0004% for 3 days caused a signficant increase in the SLRL mutation frequency compared with the solvent control group. Diallate appears to be a weak mutagen, perhaps producing its most severe effects on meiotic cells.

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*Lee, W. R., personal communication.

Table 153

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

DIALLATE

Experiment 1

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate									
			TA1535		TA1537		TA1538		TA98		TA100	
Negative Control												
DMSO	-	50.0 µl	29	27	16	12	21	17	33	26	128	155
	+	50.0	38	30	31	29	30	30	39	56	158	140
Positive Controls												
Sodium Azide	-	0.5 µg	375	398								
	-	1.0									564	537
9-Aminoacridine	-	50.0			373	415						
2-Nitrofluorene	-	5.0					824	908	429	420		
2-Anthramine	-	1.0					18	26	33	40	143	126
	+	1.0					402	514	341	400	709	759
	-	2.5	30	33	20	18						
	+	2.5	259	253	128	128						
Diallate	-	0.0005 µl	41	36	12	14	14	12	27	32	108	142
	-	0.001	29	38	16	9	16	16	30	31	100	148
	-	0.005	40	31	13	19	13	13	30	21	128	134
	-	0.01	36	39	15	17	20	9	29	30	130	164
	-	0.05	37	44	13	12	14	21	30	40	141	130
	-	0.1	37	24	15	6	16	21	31	26	100	123
	+	0.0005 µl	25	28	31	29	30	31	42	38	155	141
	+	0.001	37	38	32	30	28	33	50	50	196	194
	+	0.005	55	62	33	21	31	17	44	42	234	262
	+	0.01	128	153	36	29	29	28	64	52	500	478
	+	0.05	346	395	32	28	33	38	67	85	1018	1160
	+	0.1	622	694	21	32	27	32	93	127	1454	1579

Table 154

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

DIALLATE

Experiment 2

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate									
			TA1535		TA1537		TA1538		TA98		TA100	
Negative Control												
DMSO	-	50.0 µl	18	17	9	7	13	15	19	21	87	96
	+	50.0	19	10	25	21	21	26	36	43	96	88
Positive Controls												
Sodium Azide	-	0.5 µg	292	260								
	-	1.0									481	564
9-Aminoacridine	-	50.0			218	247						
2-Nitrofluorene	-	5.0					733	749	341	378		
2-Anthramine	-	1.0					13	18	15	28	115	113
	+	1.0					409	374	349	328	422	579
	-	2.5	28	19	15	15						
	+	2.5	261	288	152	196						
Diallate	-	0.0005 µl	29	33	12	18	18	12	19	26	110	125
	-	0.001	30	26	12	10	13	10	30	18	110	137
	-	0.005	32	30	17	12	13	10	32	36	112	124
	-	0.01	30	36	18	14	17	17	28	24	113	123
	-	0.05	30	29	14	14	18	20	20	19	154	124
	-	0.1	38	31	15	16	19	10	20	25	104	114
	+	0.0005 µl	29	18	31	26	19	28	27	41	134	129
	+	0.001	36	28	37	31	30	19	38	38	203	237
	+	0.005	76	60	36	30	36	18	50	30	262	257
	+	0.01	154	102	39	39	37	37	57	48	465	411
	+	0.05	382	408	39	31	29	32	74	67	1104	1075
	+	0.1	586	804	30	32	25	31	109	108	785	265

Table 155

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D7

DIALLATE

Experiment 1

			Survivors		Crossing-Over				Gene Conversion		Reverse Mutation	
					Mitotic Recombinants		Total Aberrants		Convertants		Revertants	
Compound	MA	% Conc.	Cells Per ml (× 10^{-7})	%	Per ml (× 10^{-3})	Per 10^5 Survivors	Per ml (× 10^{-3})	Per 10^5 Survivors	Per ml (× 10^{-1})	Per 10^6 Survivors	Per ml (× 10^0)	Per 10^7 Survivors
Negative Control												
DMSO	-	10.0	5.2	100	4.5	8.7	6.5	13	70	13	14	2.7
	+	10.0	5.6	100	1.5	2.7	11	20	66	12	16	2.9
Positive Control												
1,2,3,4-Diepoxybutane	-	0.013	3.9	75	96	250	250	640	1900	490	280	72
	+	0.013	3.7	66	92	250	370	1000	1600	430	330	89
Diallate	-	0.001	5.6	108	1.0	1.8	13	23	69	12	14	2.5
	-	0.005	5.5	106	0	0	10	18	53	9.6	9.0	1.6
	-	0.0075	2.1	40	2.0	9.5	14	6.7	15	7.1	1.0	0.48
	-	0.01	0.82	16	3.0	37	10	120	13	16	0	0
	-	0.025	T*		T		T		T		T	
	+	0.001	6.4	114	0	0	13	20	58	9.1	10	1.6
	+	0.005	6.4	114	3.0	4.7	16	25	90	14	18	2.8
	+	0.0075	4.0	71	0	0	21	53	49	12	4.0	1.0
	+	0.01	2.2	39	5.0	23	9.0	41	81	37	3.0	1.4
	+	0.025	0.63	11	9.0	140	11	170	30	48	T	

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[*] T = toxic

Table 156

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D7

DIALLATE

Experiment 2

Compound	MA	% Conc.	Survivors		Crossing-Over				Gene Conversion		Reverse Mutation	
					Mitotic Recombinants		Total Aberrants		Convertants		Revertants	
			Cells Per ml ($\times 10^{-7}$)	%	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors	Per ml ($\times 10^{-1}$)	Per 10^6 Survivors	Per ml ($\times 10^{0}$)	Per 10^7 Survivors
Negative Control												
DMSO	-	10.0	3.6	100	0	0	8.0	22	22	6.1	6.0	1.7
	+	10.0	3.7	100	0	0	8.0	22	45	12	4.0	1.1
Positive Control												
1,2,3,4-Diepoxybutane	-	0.013	1.9	53	48	250	340	1800	1100	580	150	79
	+	0.013	2.3	62	25	110	290	1300	1200	520	160	70
Diallate	-	0.00075	3.0	83	0	0	12	40	19	6.3	3.0	1.0
	-	0.001	3.6	100	2.0	5.6	13	36	19	5.3	8.0	2.2
	-	0.0025	1.4	39	0	0	9.0	64	12	8.6	5.0	3.6
	-	0.005	0.45	13	0	0	5.0	110	6.0	13	1.0	2.2
	-	0.0075	0.38	11	0	0	0	0	7.0	18	2.0	5.3
	+	0.00075	3.1	84	0	0	12	39	38	12	7.0	2.3
	+	0.001	2.7	73	0	0	7.0	26	23	8.5	4.0	1.5
	+	0.0025	2.0	54	1.0	5.0	4.0	20	15	7.5	3.0	1.5
	+	0.005	1.5	41	1.0	6.7	11	73	15	10	1.0	0.67
	+	0.0075	0.72	19	1.0	1.4	7.0	97	8.0	11	3.0	4.2

Table 157

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D7

DIALLATE

Experiment 3

			Survivors		Crossing-Over				Gene Conversion		Reverse Mutation	
					Mitotic Recombinants		Total Aberrants		Convertants		Revertants	
Compound	MA	% Conc.	Cells Per ml (× 10^{-7})	%	Per ml (× 10^{-3})	Per 10^5 Survivors	Per ml (× 10^{-3})	Per 10^5 Survivors	Per ml (× 10^{-1})	Per 10^6 Survivors	Per ml (× 10^0)	Per 10^7 Survivors
Negative Control												
DMSO	-	10.0	7.7	100	1.0	1.3	13	17	80	10	18	2.3
	+	10.0	8.4	100	0	0	13	15	72	8.6	28	3.3
Positive Control												
1,2,3,4-Diepoxybutane	-	0.013	3.8	49	120	320	580	1500	1400	370	83	22
	+	0.013	6.3	75	93	150	600	950	2300	370	160	25
Diallate	-	0.0005	5.9	77	0	0	6.0	10	77	13	21	3.6
	-	0.00075	8.0	103	1.0	1.3	11	14	71	8.9	29	3.6
	-	0.001	6.7	87	1.0	1.5	19	28	84	13	24	3.6
	-	0.002	6.0	78	2.5	4.2	25	42	55	9.2	21	3.5
	-	0.003	5.3	69	5.0	9.4	18	34	55	10	17	3.2
	+	0.0005	7.4	88	0	0	6.0	8.1	74	10	20	2.7
	+	0.00075	6.6	79	2.0	3.0	13	2.0	76	12	14	2.1
	+	0.001	8.0	95	3.0	3.8	20	25	97	12	19	2.4
	+	0.002	8.2	98	1.0	1.2	19	23	77	9.4	29	3.5
	+	0.003	6.3	75	2.0	3.2	18	29	87	14	22	3.5

Table 158

MOUSE LYMPHOMA FORWARD MUTATION CONCENTRATION-RANGE ASSAY OF DIALLATE IN THE ABSENCE OF METABOLIC ACTIVATION

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Calculated Mutation Frequency (X 10^{-6})	Induced Mutation Frequency (X 10^{-6})
Solvent control (1% DMSO)	100.0 100.0	600 600	509 516	76 76	99.3 100.7	99.3 100.7	30 29	-- --
Positive control (500 μg/ml EMS)	83.4 64.0	600 600	309 355	887 876	60.3 69.3	50.3 44.3	574 494	544 464
Diallate								
0.5	100.5	600	496	118	96.8	97.3	48	18
1	73.4	600	492	103	96.0	70.6	42	12
2	91.1	600	535	89	104.4	95.1	33	3
5	58.7	600	476	98	92.9	54.5	41	11
8	83.1	600	513	79	100.1	83.2	31	1
10	91.6	600	522	90	101.9	93.3	34	4
20	75.6	600	494	96	96.4	72.9	39	9
30	59.3	600	583	172	113.8	67.5	59	29
50	45.1	600	455	109	88.8	40.0	48	18
60	16.2	600	418	161	81.6	13.2	77	47
80	1.4	NC*	--	--	--	--	--	--
100	0.2	NC	--	--	--	--	--	--
150	0.1	NC	--	--	--	--	--	--
200	0.1	NC	--	--	--	--	--	--
250	0.1	NC	--	--	--	--	--	--
300	0.2	NC	--	--	--	--	--	--
400	0.1	NC	--	--	--	--	--	--
500	0.1	NC	--	--	--	--	--	--

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*Not cloned.

Table 159

MOUSE LYMPHOMA FORWARD MUTATION CONCENTRATION-RANGE ASSAY OF DIALLATE IN THE PRESENCE OF INDUCED METABOLIC ACTIVATION

Concentration (µg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Calculated Mutation Frequency (X 10^{-6})	Induced Mutation Frequency (X 10^{-6})
Solvent control (1% DMSO)	102.3 97.7	600 600	538 536	298 274	100.2 99.8	102.5 97.5	111 102	-- --
Positive control (4.0 µg/ml 3-MCA)	71.4 74.5	600 600	373 411	488 492	69.5 76.5	49.6 57.0	262 239	155 132
Diallate								
0.5	104.1	600	530	260	98.7	102.7	98	
1	109.1	600	524	299	97.6	106.5	114	--
2	104.1	600	518	295	96.5	100.4	114	7
5	83.5	600	502	338	93.5	78.1	135	7
8	53.7	600	435	343	81.0	43.5	158	28
10	33.9	600	392	407	73.0	24.7	208	51
20	4.1	NC*	--	--	--	--	--	101
30	0.7	NC	--	--	--	--	--	--
50	0.3	NC	--	--	--	--	--	--
60	0.4	NC	--	--	--	--	--	--
80	0.4	NC	--	--	--	--	--	--
100	0.3	NC	--	--	--	--	--	--
150	0.5	NC	--	--	--	--	--	--
200	0.5	NC	--	--	--	--	--	--
250	0.6	NC	--	--	--	--	--	--
300	0.4	NC	--	--	--	--	--	--
400	0.4	NC	--	--	--	--	--	--
500	0.6	NC	--	--	--	--	--	--

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*Not cloned.

Table 160

MOUSE LYMPHOMA FORWARD MUTATION ASSAY OF DIALLATE IN THE ABSENCE OF METABOLIC ACTIVATION

Concentration (µg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Calculated Mutation Frequency (X 10⁻⁶)	Induced Mutation Frequency (X 10⁻⁶)
Solvent Control (1% DMSO)	100.0 100.0	600 600	519 494	153 105	102.5 97.5	102.5 97.5	59 43	-- --
Positive Control (500 µg/ml EMS)	77.2 77.2	600 600	391 355	937 926	77.2 70.1	60.0 54.1	479 522	428 471
Diallate								
4	94.5 85.1	600 600	589 515	123 76	116.3 101.7	109.9 86.5	42 30	-- --
12	80.0 85.2	600 600	470 524	91 119	92.8 103.5	74.2 88.1	39 45	-- --
20	62.4 65.3	600 600	556 525	130 125	109.8 103.7	68.5 67.7	47 48	-- --
32	50.9 55.1	600 600	519 560	107 102	102.5 110.6	52.2 60.9	41 36	-- --
40	42.2 45.3	600 600	569 550	167 161	112.3 108.6	47.4 49.2	59 59	8 8
52	31.3 36.7	600 600	534 482	182 194	105.4 95.2	33.0 34.9	68 80	17 29
56	20.4 22.0	600 600	507 481	156 149	100.1 95.0	20.4 20.9	62 62	11 11
60	12.6 18.6	600 600	384 462	186 154	75.8 91.2	9.6 17.0	97 67	46 16
72	1.0 3.1	NC* 600	-- 100	-- 150	-- 19.7	-- 0.6	-- 300	-- 249
80	1.7 1.5	NC NC	-- --	-- --	-- --	-- --	-- --	-- --

* Not Cloned

Table 161

REPEAT OF MOUSE LYMPHOMA FORWARD MUTATION ASSAY OF DIALLATE IN THE ABSENCE OF METABOLIC ACTIVATION

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Calculated Mutation Frequency (X 10^{-6})	Induced Mutation Frequency (X 10^{-6})
Solvent control (1% DMSO)	118.5	600	587	87	100.3	118.8	30	--
	81.5	600	584	94	99.7	81.3	32	--
Positive control (500 μg/ml EMS)	77.2	600	340	974	58.1	44.8	573	542
	54.7	600	367	936	62.7	34.3	510	479
Diallate								
4.8	104.5	600	542	133	92.6	96.7	49	18
	99.2	600	553	99	94.4	93.7	36	5
12	105.5	600	546	108	93.3	98.4	40	9
	105.5	600	557	102	95.1	100.4	37	6
20	77.2	600	508	136	86.8	67.0	54	23
	73.1	600	485	142	82.8	60.6	59	28
32	53.9	600	425	152	72.6	39.1	72	41
	52.1	600	463	117	79.1	41.2	51	20
40	48.9	600	540	160	92.2	45.1	59	28
	41.7	600	497	121	84.9	35.4	49	18
52	38.0	600	548	130	93.6	35.6	47	16
	33.8	600	510	124	87.1	29.4	49	18
56	29.9	600	469	164	80.1	23.9	70	39
	28.5	600	471	153	80.4	22.9	65	34
60	11.6	600	383	163	65.4	7.6	85	54
	15.9	600	431	183	73.6	11.7	85	54
72	2.3	NC*	--	--	--	--	--	--
	2.6	NC	--	--	--	--	--	--
80	1.3	NC	--	--	--	--	--	--
	1.7	NC	--	--	--	--	--	--

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*Not cloned.

Table 162

MOUSE LYMPHOMA FORWARD MUTATION ASSAY OF DIALLATE IN THE PRESENCE OF INDUCED METABOLIC ACTIVATION

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Calculated Mutation Frequency (X 10⁻⁶)	Induced Mutation Frequency (X 10⁻⁶)
Solvent control	91.4	600	621	188	108.5	99.1	61	--
(1% DMSO)	108.6	600	524	125	91.5	99.4	48	--
Positive control	59.1	600	385	557	67.2	39.7	289	234
(4.0 μg/ml 3-MCA)	68.0	600	362	476	63.2	43.0	263	208
Diallate								
1	114.0	600	556	144	97.1	110.7	52	--
	107.6	600	577	184	100.8	108.4	64	9
5	91.4	600	601	229	105.0	96.0	76	21
	112.4	600	861	277	150.4	169.0	64	9
6	91.4	600	615	249	107.4	98.2	81	26
	93.1	600	529	219	92.4	86.0	83	28
7	98.7	600	565	256	98.7	97.4	91	36
	104.8	600	597	244	104.3	109.3	82	27
8	88.3	600	530	209	92.6	81.7	79	24
	80.5	600	398	178	69.5	56.0	89	34
9	75.6	600	597	277	104.3	78.8	93	38
	78.0	600	499	295	87.2	68.0	118	63
10	71.7	600	532	286	92.9	66.6	108	53
	77.8	600	554	272	96.8	75.3	98	43
15	37.6	600	554	295	96.8	36.4	107	52
	37.5	600	494	292	86.3	32.4	118	63
18	30.4	600	485	381	84.7	25.8	157	102
	27.7	600	416	297	72.7	20.1	143	88
20	24.6	600	377	333	65.9	16.2	177	122
	22.8	600	441	320	77.0	17.6	145	90

Table 163

REPEAT OF MOUSE LYMPHOMA FORWARD MUTATION ASSAY OF DIALLATE IN THE PRESENCE OF INDUCED METABOLIC ACTIVATION

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Calculated Mutation Frequency ($X 10^{-6}$)	Induced Mutation Frequency ($X 10^{-6}$)
Solvent control (1% DMSO)	120.0	600	459	201	98.5	118.2	88	--
	80.0	600	473	233	101.5	81.2	99	--
Positive control (4.0 μg/ml 3-MCA)	61.6	600	354	537	76.0	46.8	303	209
	48.6	600	358	538	76.8	37.3	301	207
Diallate								
7	70.4	600	441	312	94.6	66.6	141	47
	68.6	600	421	306	90.3	62.0	145	51
8	65.2	600	442	223	94.8	61.8	101	7
	70.4	600	426	272	91.4	64.4	128	34
9	62.1	600	408	348	87.6	54.4	171	77
	67.0	600	414	290	88.8	59.5	140	46
10	57.9	600	397	308	85.2	49.3	155	61
	57.9	600	442	261	94.8	54.9	118	24
12	34.7	600	304	234	65.2	22.6	154	60
	49.9	600	477	333	102.4	51.1	140	46
14	37.0	600	364	323	78.1	28.9	177	83
	39.3	600	386	336	82.8	32.6	174	80
16	30.8	600	334	333	71.7	22.1	199	105
	31.6	600	413	376	88.6	28.0	182	88
18	21.8	600	330	333	70.8	15.4	202	108
	23.8	600	295	415	63.3	15.1	281	187
20	20.8	600	265	390	56.9	11.8	294	200
	19.5	600	278	339	59.7	11.6	244	150
25	13.7	600	217	373	46.6	6.4	344	250
	15.7	600	244	401	52.4	8.2	329	235

Table 164

EFFECT OF DIALLATE ON VIABILITY AND FERTILITY OF *DROSOPHILA* MELANOGASTER MALES

Concentrations of Diallate/Day (%)	Viability (%)	Fertility (%)
Wide-Range Pretest		
0	100	90
0.000000319[*]	70	100
0.00000169[*]	100	100
0.00000876[*]	80	100
0.0000482[*]	100	89[†]
0.000282[*]	90	89
0.00177[*]	50	80
0.0122[*]	0	--
0.0905[*]	0	--
Narrow-Range Pretest		
0	100	100
0.0000313	80	100
0.0000625	90	89
0.000125	100	100
0.00025	90	100
0.0005	40	75
0.001	0	--
0.002	0	--
0.004	0	--

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[*] Average daily dose because of variations resulting from dilution errors.

[†] One male escaped before mating.

Table 165

NUMBERS OF SEX-LINKED RECESSIVE LETHAL MUTATIONS IN DROSOPHILA MELANOGASTER MALES FED DIALLATE FOR 3 DAYS

Treatment	Experiment No.	No. of Lethals/Total Sample Size per Brood A	B	C	Total Sample Size	Total No. Lethals
Solvent control (1% DMSO)	I	2/602	2/993	0/187	1782	4
	II	2/1183	3/427	0/248	1858	5
	III	4/2491	7/2263	4/413	5167	15[*]
	All	8/4276	12/3683	4/848	8807	24
Diallate (0.0004%)	I	4/1116	2/913	12/1652	3681	18[†]
	II	5/1000	--	--	1000	5
	III	4/1736	6/1571	8/828	4135	18[‡]
	All	13/3852	8/2484	20/2480	8816	41
Positive Control (0.01% EMS)	I	7/52	2/47	4/37	136	13[§]
	II	8/53	4/56	9/54	163	21[**]
	III	23/49	16/47	4/21	117	43[††]
	All	38/154	22/150	17/112	416	77

Note = -- = No results.

---

[*] Includes 1 cluster of 2 and 1 cluster of 3; excludes 1 cluster of 7.

[†] Includes 3 clusters of 2.

[‡] Includes 2 clusters of 2.

[§] Includes 3 clusters of 2 and 1 cluster of 3.

[**] Includes 4 clusters of 2, 1 cluster of 3, 1 cluster of 4, and 1 cluster of 6.

[††] Includes 1 cluster of 2, 2 clusters of 3, 2 clusters of 4, 1 cluster of 5, 2 clusters of 6, and 1 cluster of 10.

Table 166

SEX-LINKED RECESSIVE LETHAL MUTATION FREQUENCIES IN *DROSOPHILA MELANOGASTER* MALES FED DIALLATE FOR 3 DAYS

		Mutation Frequency (%)			
		Brood			All
Treatment	Experiment No.	A	B	C	Broods
Solvent control (1% DMSO)	I	0.33	0.20	0	0.22
	II	0.17	0.70	0	0.27
	III	0.16	0.31	0.97	0.29
	All	0.19	0.32	0.47	0.27
Diallate (0.0004%)	I	0.36	0.22	0.73	0.49
	II	0.50	--*	--*	0.50
	III	0.23	0.38	0.97	0.44
	All	0.34	0.32	0.81	0.47
Positive control (0.01% EMS)	I	13.5	4.3	10.8	9.6
	II	15.1	7.1	16.7	12.9
	III	46.9	34.0	19.0	36.8
	All	24.7	14.7	15.2	18.5

---

*No results.

Dicamba

S. typhimurium Plate Incorporation, E. coli WP-2, S. cerevisiae D3. Dicamba was previously assayed in these test systems, and was found to be negative in all three, both with and without activation (52).

E.coli/B. subtilis Relative Toxicity. This compound was found to be positive in both species in previous testing (52).

S. typhimurium Relative Toxicity (Tables 167, 168). Dicamba was negative in both strain pairs in this assay.

UDS in WI-38 Cells. This compound was previously tested and found to be negative, both with and without activation (52).

Table 167

DIFFERENTIAL TOXICITY ASSAYS WITH SALMONELLA TYPHIMURIUM STRAINS

Experiment 1

Compound	Amount of Compound Applied to Disc	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		SL4525 (rec^+)		SL4700 (rec^-)		TA1978 ($uvrB^+$)		TA1538 ($uvrB^-$)	
Negative Control									
Kanamycin	100.0 μg	14	13	13	14	13	13	14	14
Positive Controls									
MMS*	10.0 mg	47	46	68	65				
4-NQO†	2.0 mg					12	12	24	23
Dicamba	1.0 mg	20	22	20	19	17	17	14	16
	5.0 mg	28	25	25	26	22	21	21	22

---

* MMS = Methylmethanesulfonate.

† 4-NQO = 4-Nitroquinoline-1-oxide.

Table 168

DIFFERENTIAL TOXICITY ASSAYS WITH SALMONELLA TYPHIMURIUM STRAINS

Experiment 2

Compound	Amount of Compound Applied to Disc	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		SL4525 (rec^+)		SL4700 (rec^-)		TA1978 ($uvrB^+$)		TA1538 ($uvrB^-$)	
Negative Control									
Kanamycin	**100.0 µg**	**15**	**14**	**16**	**15**	**17**	**17**	**15**	**16**
Positive Controls									
MMS*	**10.0 mg**	45	40	72	74				
4-NQO†	**2.0 mg**					**12**	**12**	**28**	**26**
Dicamba	1.0 mg	22	20	19	19	11	10	15	14
	5.0 mg	23	22	23	24	25	23	22	24

---

* MMS = Methylmethanesulfonate.

† 4-NQO = 4-Nitroquinoline-1-oxide.

m-Dichlorobenzene

S. typhimurium Plate Incorporation, E. coli WP-2 (Tables 169, 170). The compound was tested over a range of 0.05 to 1.0 µℓ/plate (~ 0.065 to 1.3 mg/plate; density ≈ 1.3) and retested at lower levels ranging from 0.0005 to 0.5 µℓ/plate (~ 0.00065 to 0.65 mg/plate). Both experiments were conducted in duplicate on separate days. *m*-Dichlorobenzene was toxic to all strains at 1.0 µℓ/plate with and without metabolic activation. No reproducible dose-related increase in the number of revertants was observed with *m*-dichlorobenzene.

S. cerevisiae D3 (Tables 171, 172). A reproducible increase in the number of mitotic recombinants per 10^5 survivors was observed with and without metabolic activation. The compound was tested at concentrations ranging from 0.0005 to 0.0075% and retested over a range of 0.001 to 0.01%. In both experiments, metabolic activation appeared to enhance the recombinogenic response.

E. coli/B. subtilis Relative Toxicity (Table 173). In four of the five experiments, this compound was more toxic to the *E. coli polA*$^-$ strain p3478 than to the *polA*$^+$ strain W3110. This compound caused two zones of inhibition of growth to both *E. coli* strains. The zone recorded in Table 173 was the inner clear zone immediately surrounding the disc containing the compound. A second clear zone, concentric to the first, was observed in all five experiments with the *polA*$^+$ and the *polA*$^-$ strains. This unusual toxicity pattern along a concentration gradient, where a high concentration was toxic, a medium concentration was not toxic, and a low concentration was toxic, might suggest saturation of a critical uptake system or metabolic pathway. *m*-Dichlorobenzene was equally toxic to both the *rec*$^-$ and *rec*$^+$ *B. subtilis* strains. These results indicate that *m*-dichlorobenzene causes DNA damage in the *E. coli polA*$^+$/*polA*$^-$ assay but does not in the *B. subtilis rec*$^+$/*rec*$^-$ assay.

Table 169

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI WP2
m-Dichlorobenzene
Experiment 1

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate										Tryptophan Revertants per Plate	
			TA1535		TA1537		TA1538		TA98		TA100		WP2	
Negative control														
DMSO	-		32	20	8	12	18	14	28	29	104	91	33	39
	+		21	26	14	15	16	24	29	39	95	108	47	45
Positive controls														
Sodium azide	-	0.5 µg	296	344										
	-	1.0									470	581		
9-Aminoacridine	-	50.0			144	203								
2-Nitrofluorene	-	5.0					760	661	434	542				
AF2	-	0.1											940	922
2-Anthramine	-	1.0					25	21	24	27	116	104		
	+	1.0					375	372	297	300	411	392		
	-	2.5	32	33	12	14								
	+	2.5	222	246	77	79								
	-	10.0											39	41
	+	10.0											218	238
m-Dichlorobenzene	-	0.05 µl	13	26	7	13	14	16	24	20	83	102	21	41
	-	0.10	25	26	8	10	17	13	21	19	81	100	34	44
	-	0.25	22	27	6	9	15	20	18	20	87	100	26	29
	-	0.50	23	11	2	3	16	7	17	11	97	85	32	36
	-	0.75	24	19	3	5	9	15	19	22	84	T*	32	29
	-	1.00	T	T	T	T	T	T	T	T	T	T	T	T
	+	0.05	19	18	15	16	36	32	29	40	108	91	38	52
	+	0.10	14	14	9	5	30	27	30	41	101	120	52	48
	+	0.25	18	14	18	7	26	25	36	36	100	89	49	53
	+	0.50	13	9	8	10	26	19	29	38	111	87	50	50
	+	0.75	15	9	9	5	17	24	34	29	99	65	49	38
	+	1.00	T	T	T	T	T	T	T	T	T	T	T	T

---

*T, toxic.

Table 170

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI WP2
m-Dichlorobenzene
Experiment 2

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate										Tryptophan Revertants per Plate	
			TA1535		TA1537		TA1538		TA98		TA100		WP2	
Negative control														
DMSO	-		30	23	6	7	18	14	14	16	77	78	48	46
	+		6	8	11	14	24	35	32	29	89	85	46	53
Positive controls														
Sodium azide	-	0.5 µg	249	263										
	-	1.0									170	191		
9-Aminoacridine	-	50.0			114	69								
2-Nitrofluorene	-	5.0					834	875	674	533				
AF2	-	0.1											1805	1880
2-Anthramine	-	1.0					18	38	18	20	75	85		
	+	1.0					622	465	654	641	569	527		
	-	2.5	20	21	12	8								
	+	2.5	345	324	140	167								
	-	10.0											53	55
	+	10.0											1115	1008
m-Dichlorobenzene	-	0.0005 µl	17	26	7	4	11	20	15	28	78	95	49	53
	-	0.0010	17	29	8	9	17	16	20	22	74	121	42	39
	-	0.0100	17	13	3	8	17	17	19	21	83	97	39	40
	-	0.0500	20	17	6	3	17	10	19	20	91	89	38	40
	-	0.1000	12	11	6	3	14	15	17	15	91	99	40	50
	-	0.5000	17	12	4	8	11	3	20	10	64	61	41	36
	+	0.0005	5	10	14	13	33	41	21	37	87	100	49	42
	+	0.0010	12	9	14	10	30	37	29	30	85	78	50	39
	+	0.0100	10	7	14	11	27	42	42	36	78	93	44	42
	+	0.0500	13	9	15	15	32	39	32	27	81	101	45	45
	+	0.1000	9	10	7	8	39	32	29	39	76	85	41	55
	+	0.5000	8	9	11	8	20	18	24	31	82	85	49	46

Table 171

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

m-Dichlorobenzene
Experiment 1

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml (x 10^{-7})	Percent	Per ml (x 10^{-3})	Per 10^5 Survivors
Negative control DMSO	–		4.7	100	3.5	7.5
	+		6.1	100	1.5	2.5
Positive control 1,2,3,4-Diepoxybutane	–	0.025	4.9	104	913	1900
	+	0.025	4.2	69	723	1700
m-Dichlorobenzene	–	0.0005	6.7	143	6.0	9.0
	–	0.0010	5.7	121	4.0	7.0
	–	0.0025	6.2	132	9.0	15
	–	0.0050	5.9	126	9.0	15
	–	0.0075	3.8	81	9.0	24
	+	0.0005	6.2	102	9.0	15
	+	0.0010	5.4	89	4.0	7.4
	+	0.0025	4.0	66	8.0	20
	+	0.0050	5.3	87	11.0	21
	+	0.0075	1.9	31	5.0	26

Table 172

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
m-Dichlorobenzene
Experiment 2

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control						
DMSO	-		6.3	100	3.5	5.6
	+		7.9	100	2.0	2.5
Positive control						
1,2,3,4-Diepoxybutane	-	0.025	6.1	97	708	1200
	+	0.025	6.3	80	883	1400
m-Dichlorobenzene	-	0.0010	4.8	76	5.0	10
	-	0.0025	6.6	105	8.0	12
	-	0.0050	6.0	95	7.0	12
	-	0.0075	6.7	106	7.0	10
	-	0.0100	6.5	103	6.0	9.2
	+	0.0010	4.7	59	7.0	15
	+	0.0025	5.2	66	5.0	9.6
	+	0.0050	5.1	65	12.0	24
	+	0.0075	5.3	67	7.0	13
	+	0.0100	4.9	62	6.0	12

Table 173

DIFFERENTIAL TOXICITY ASSAYS
m-Dichlorobenzene

		Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		E. coli				B. subtilis			
Compound	Amount	W3110 (polA$^+$)		p3478 (polA$^-$)		H17 (rec$^+$)		M45 (rec$^-$)	
EXPERIMENT 1									
Negative control									
Ampicillin	0.01 mg	20	20	18	17	25	26	28	27
Positive control									
Methylmethane sulfonate	1.0	30	30	35	35	14	14	49	46
m-Dichlorobenzene	10.0 µl	12	11	12	12	10	10	11	10
	20.0	12	12	18	18	13	13	14	13
EXPERIMENT 2									
Negative control									
Ampicillin	0.01 mg		18		16		11		11
Positive control									
Methylmethane sulfonate	1.0		30		39		9		49
m-Dichlorobenzene	10.0 µl	11	11	11	11	10	10	10	10
	20.0	13	13	13	13	12	11	13	12
EXPERIMENT 3									
Negative control									
Ampicillin	0.01 mg	19	18	17	17	25	25	27	27
Positive control									
Methylmethane sulfonate	1.0	16	16	22	23	15	15	50	50
m-Dichlorobenzene	20.0 µl	12	12	15	17	13	14	15	15
EXPERIMENT 4									
Negative control									
Ampicillin	0.01 mg	18	18	16	17	*	*	*	*
Positive control									
Methylmethane sulfonate	1.0	14	15	31	29	*	*	*	*
m-Dichlorobenzene	20.0 µl	16	15	17	19	*	*	*	*
EXPERIMENT 5									
Negative control									
Ampicillin	0.01 mg	18	19	17	18	18	21	21	22
Positive control									
Methylmethane sulfonate	1.0	16	13	23	20	10	12	50	48
m-Dichlorobenzene	20.0 µl	15	13	18	17	12	12	12	13

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*Controls not acceptable

o-Dichlorobenzene

S. typhimurium Plate Incorporation, E. coli WP-2 (Tables 174, 175). All experimental procedures with *o*-dichlorobenzene included measures to protect the chemical from exposure to light. In assays with *S. typhimurium* and *E. coli* WP2, the compound did not demonstrate a reproducible dose-related increase in the number of revertants with or without activation. *o*-Dichlorobenzene was tested once over a concentration range of 0.05 to 1.0 μℓ/plate (~ 0.065 to 1.3 mg/plate; density ≈ 1.3) and retested at a lower range from 0.0005 to 0.5 μℓ/plate (~ 0.00065 to 0.65 mg/plate). Table 11 shows TA100 to be more sensitive to the toxic effects of *o*-dichlorobenzene, exhibiting pinpoint colonies and a thinning of the background lawn at 0.5 μℓ/plate. Toxicity was observed at 1.0 μℓ/plate for the other *S. typhimurium* strains and for *E. coli* WP2.

S. cerevisiae D3 (Tables 176-178). No recombinogenic activity was observed in the *S. cerevisiae* D3 assays with *o*-dichlorobenzene. The compound was tested once at concentrations ranging from 0.001 to 0.01% and retested over a range of 0.001 to 0.025%. Toxicity was observed at 0.05% with and without metabolic activation (Table 176).

E. coli/B. subtilis Relative Toxicity (Table 179). In three of the four experiments, the compound was reproducibly more toxic to the repair-deficient *E. coli polA*$^-$ strain p3478 than to the repair-proficient *E. coli polA*$^+$ strain W3110. Double concentric zones of toxicity, similar to those seen in the differential toxicity assays with *m*-dichlorobenzene were observed. There was no reproducible difference in the toxicity of *o* dichlorobenzene to the *rec*$^-$ and *rec*$^+$ *B. subtilis* strains. These findings indicate that *o*-dichlorobenzene is capable of interacting with and damaging bacterial DNA in the *polA*$^+$/*polA*$^-$ *E. coli* strains, but not in the *rec*$^+$/*rec*$^-$ *B. subtilis* strains.

Table 174

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI WP2
o-Dichlorobenzene
Experiment 1

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate										Tryptophan Revertants per Plate	
			TA1535		TA1537		TA1538		TA98		TA100		WP2	
Negative control														
DMSO	-		32	20	8	12	18	14	28	29	104	91	33	39
	+		21	26	14	15	16	24	29	39	95	108	47	45
Positive controls														
Sodium azide	-	0.5 µg	296	344										
	-	1.0									470	581		
9-Aminoacridine	-	50.0			144	203								
2-Nitrofluorene	-	5.0					760	661	434	542				
AF2	-	0.1											940	922
2-Anthramine	-	1.0					25	21	24	27	116	104		
	+	1.0					375	372	297	300	411	392		
	-	2.5	32	33	12	14								
	+	2.5	222	246	77	79								
	-	10.0											39	41
	+	10.0											218	238
o-Dichlorobenzene	-	0.05 µl	28	31	12	8	16	18	27	24	96	99	25	50
	-	0.10	17	30	8	9	13	11	25	32	109	96	29	29
	-	0.25	21	26	8	9	14	15	26	22	100	100	28	29
	-	0.50	24	20	5	5	18	9	19	18	T	T*	27	36
	-	0.75	17	16	1	3	11	10	11	11	T	T	31	36
	-	1.00	[illegible]	T	T	T	T	T	T	T	T	T	T	T
	+	0.05	17	18	13	14	18	26	34	36	94	86	44	48
	+	0.10	18	16	16	15	22	31	28	35	109	110	52	50
	+	0.25	14	16	13	17	22	26	31	37	103	78	46	43
	+	0.50	19	7	18	13	26	25	34	27	58	93	41	36
	+	0.75	10	9	15	15	26	26	C†	21	T	T	55	38
	+	1.00	T	T	T	T	T	T	T	T	T	T	T	T

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*T, toxic.

†C, contamination.

Table 175

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI WP2
o-Dichlorobenzene
Experiment 2

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate										Tryptophan Revertants per Plate	
			TA1535		TA1537		TA1538		TA98		TA100		WP2	
Negative control														
DMSO	-		30	23	6	7	18	14	14	16	77	78	48	46
	+		6	8	11	14	24	35	32	29	89	85	46	53
Positive controls														
Sodium azide	-	0.5 µg	249	263										
	-	1.0									170	191		
9-Aminoacridine	-	50.0			114	69								
2-Nitrofluorene	-	5.0					834	875	674	533				
AF2	-	0.1											1805	1880
2-Anthramine	-	1.0					18	38	18	20	75	85		
	+	1.0					622	465	654	641	569	527		
	-	2.5	20	21	12	8								
	+	2.5	345	324	140	167								
	-	10.0											53	55
	+	10.0											1115	1008
o-Dichlorobenzene	-	0.0005 µl	21	22	4	6	20	16	21	26	99	93	41	39
	-	0.0010	15	18	9	4	15	17	21	22	98	101	31	36
	-	0.0100	23	16	3	6	15	14	20	19	100	91	37	37
	-	0.0500	21	17	8	4	17	15	18	19	96	103	31	41
	-	0.1000	21	23	8	2	18	11	27	29	76	84	53	32
	-	0.5000	17	17	4	11	8	11	19	16	74	84	36	36
	+	0.0005	15	13	17	21	31	34	34	27	98	86	51	51
	+	0.0010	9	10	11	12	18	19	18	33	113	109	48	52
	+	0.0100	8	11	9	13	31	30	38	34	83	111	57	58
	+	0.0500	13	12	8	10	23	31	28	31	108	108	54	44
	+	0.1000	17	17	13	15	25	16	35	34	103	102	42	52
	+	0.5000	11	11	15	13	18	25	35	31	108	104	50	34

Table 176

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
o-Dichlorobenzene
Experiment 1

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors: Cells per ml ($x\ 10^{-7}$)	Survivors: Percent	Mitotic Recombinants: Per ml ($x\ 10^{-3}$)	Mitotic Recombinants: Per 10^5 Survivors
Negative control DMSO	-		5.7	100	1.0	1.8
	+		6.2	100	3.0	4.8
Positive control 1,2,3,4-Diepoxybutane	-	0.025	5.7	100	583	1000
	+	0.025	6.2	100	465	750
o-Dichlorobenzene	-	0.001	7.8	137	3.0	3.9
	-	0.005	7.5	132	5.0	6.7
	-	0.01	6.8	119	3.0	4.4
	-	0.05	T*		T	
	+	0.001	9.0	145	2.0	2.2
	+	0.005	7.1	115	4.0	5.6
	+	0.01	7.5	121	3.0	4.0
	+	0.05	T		T	

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*T, toxic.

Table 177

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
o-Dichlorobenzene
Experiment 2

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control						
DMSO	-		6.1	100	4.5	7.4
	+		6.6	100	2.0	3.0
Positive control						
1,2,3,4-Diepoxybutane	-	0.025	5.8	95	373	640
	+	0.025	5.3	80	478	900
o-Dichlorobenzene	-	0.001	5.2	85	3.0	5.8
	-	0.0025	7.2	118	3.0	4.2
	-	0.005	9.2	151	3.0	3.0
	-	0.0075	4.4	72	1.0	2.3
	-	0.01	3.3	54	2.0	6.1
	+	0.001	3.5	53	1.0	2.9
	+	0.0025	5.7	86	1.0	1.8
	+	0.005	5.4	82	5.0	9.3
	+	0.0075	3.7	56	3.0	8.1
	+	0.01	3.9	59	2.0	5.1

Table 178

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
o-Dichlorobenzene
Experiment 3

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($x\ 10^{-7}$)	Percent	Per ml ($x\ 10^{-3}$)	Per 10^5 Survivors
Negative control						
DMSO	-		6.3	100	3.5	5.6
	+		7.9	100	2.0	2.5
Positive control						
1,2,3,4-Diepoxybutane	-	0.025	6.1	97	708	1200
	+	0.025	6.3	80	883	1400
o-Dichlorobenzene	-	0.001	7.5	119	6.0	8.0
	-	0.005	7.0	111	3.0	4.3
	-	0.0075	7.6	121	2.0	2.6
	-	0.01	6.8	108	5.0	7.4
	-	0.025	7.2	114	4.0	5.6
	+	0.001	5.9	75	5.0	8.5
	+	0.005	8.3	105	4.0	4.8
	+	0.01	7.4	94	5.0	6.8
	+	0.025	8.6	109	3.0	3.5

Table 179

DIFFERENTIAL TOXICITY ASSAYS
o-Dichlorobenzene

		Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		E. coli				B. subtilis			
Compound	Amount	W3110 (polA^{+})		p3478 (polA^{-})		H17 (rec^{+})		M45 (rec^{-})	
EXPERIMENT 1									
Negative control									
Ampicillin	0.01 mg		18		16		11		11
Positive control									
Methylmethane sulfonate	1.0		30		39		9		49
o-Dichlorobenzene	10.0 μl	13	12	12	12	11	11	12	11
	20.0	17	14	14	14	14	14	15	15
EXPERIMENT 2									
Negative control									
Ampicillin	0.01 mg	19	18	17	17	25	25	27	27
Positive control									
Methylmethane sulfonate	1.0	16	16	22	23	15	15	50	50
o-Dichlorobenzene	20.0 μl	16	15	19	18	18	18	20	20
EXPERIMENT 3									
Negative control									
Ampicillin	0.01 mg	18	18	16	17				
Positive control									
Methylmethane sulfonate	1.0	14	15	31	29				
o-Dichlorobenzene	20.0 μl	15	15	21	19				
EXPERIMENT 4									
Negative control									
Ampicillin	0.01 mg	18	19	17	18	18	21	21	22
Positive control									
Methylmethane sulfonate	1.0	16	13	23	20	10	12	50	48
o-Dichlorobenzene	20.0 μl	15	16	18	19	20	19	19	18

p-Dichlorobenzene

S. typhimurium Plate Incorporation, E. coli WP-2 (Tables 180, 181). The compound was tested once over a concentration range of 50 to 1000 μg/plate and retested over a range of 0.5 to 500.0 μg/plate. Toxicity was observed at 750 μg/plate in the absence of metabolic activation with TA100 and at 1000 μg/plate with all other strains. No reproducible dose-related increase in the number of revertants was observed with *p*-dichlorobenzene, with or without activation.

S. cerevisiae D3 (Tables 182-186). In the first three experiments, mitotic recombination was increased; however, there was considerable variation in survival. In two subsequent experiments, toxicity was observed but mitotic recombination was not increased. In some experiments, 95% or more toxicity was observed at the lower doses and no toxicity occurred at higher doses (e.g., Tables 182 and 183). These results may be caused by the relative insolubility of *p*-dichlorobenzene. At concentrations of 0.05% and higher, a milky white suspension was observed and precipitation occurred at 0.5% and higher. Our results are inconsistent and not reproducible; although they suggest that under some conditions *p*-dichlorobenzene may interact with the genetic material and increase mitotic recombination, we have classified this compound as negative in this system.

E. coli/B. subtilis Relative Toxicity (Table 187). This compound required slight sonification to dissolve in DMSO at a concentration of 500 mg/per ml. Little or no zone of inhibition of growth was observed with any of the repair-proficient and -deficient *E. coli* and *B. subtilis* strains exposed to *p*-dichlorobenzene. This could indicate that the compound is not toxic at the concentrations tested to any of the four strains used in the differential toxicity assay, or that the compound did not diffuse away from the filter paper disc through the aqueous agar medium. We have classified *p*-dichlorobenzene as negative in this assay.

Table 180

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI WP2
p-Dichlorobenzene
Experiment I

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate										Tryptophan Revertants per Plate	
			TA1535		TA1537		TA1538		TA98		TA100		WP2	
Negative control														
DMSO	-		32	20	8	12	18	14	28	29	104	91	33	39
	+		21	26	14	15	16	24	29	39	95	108	47	45
Positive controls														
Sodium azide	-	0.5 µg	296	344										
	-	1.0									470	581		
9-Aminoacridine	-	50.0			144	203								
2-Nitrofluorene	-	5.0					760	661	434	542				
AF2	-	0.1											940	922
2-Anthramine	-	1.0					25	21	24	27	116	104		
	+	1.0					375	372	297	300	411	392		
	-	2.5	32	33	12	14								
	+	2.5	222	246	77	79								
	-	10.0											39	41
	+	10.0											218	238
p-Dichlorobenzene	-	50	16	18	9	8	19	12	32	33	100	92	32	38
	-	100	21	21	15	4	14	25	20	21	97	98	34	32
	-	250	18	13	7	9	8	18	25	29	100	91	38	38
	-	500	14	18	4	6	10	12	25	24	90	92	33	33
	-	750	16	13	6	1	10	8	19	8	T	T*	29	31
	-	1000	T	T	T	T	T	T	T	T	T	T	T	T
	+	50	14	8	18	12	19	25	32	39	87	81	52	40
	+	100	18	14	16	17	18	30	46	38	78	81	41	45
	+	250	10	13	12	16	26	25	45	35	102	74	40	49
	+	500	20	9	7	9	18	24	21	31	91	92	33	36
	+	750	7	9	8	7	20	24	24	31	85	76	48	45
	+	1000	11	13	10	11	18	16	21	27	73	74	35	42

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[*] T, toxic.

Table 181

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI WP2
p-Dichlorobenzene
Experiment 2

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate										Tryptophan Revertants per Plate	
			TA1535		TA1537		TA1538		TA98		TA100		WP2	
Negative control														
DMSO	-		30	23	6	7	18	14	14	16	77	78	48	46
	+		6	8	11	14	24	35	32	29	89	85	46	53
Positive control														
Sodium azide	-	0.5 µg	249	263										
	-	1.0									170	191		
9-Aminoacridine	-	50.0			114	69								
2-Nitrofluorene	-	5.0					834	875	674	533				
AF2	-	0.1											1805	1880
2-Anthramine	-	1.0					18	38	18	20	75	85		
	+	1.0					622	465	654	641	569	527		
	-	2.5	20	21	12	8								
	+	2.5	345	324	140	167								
	-	10.0											53	55
	+	10.0											1115	1008
p-Dichlorobenzene	-	0.5	20	21	5	7	7	7	20	16	68	70	37	42
	-	1.0	13	13	8	5	15	11	20	19	76	67	43	37
	-	10.0	17	17	3	4	9	7	27	28	88	86	50	44
	-	50.0	15	20	6	4	15	4	15	17	85	79	50	53
	-	100.0	11	28	6	5	10	12	18	9	84	61	37	37
	-	500.0	11	17	5	1	14	6	21	9	79	40	35	30
	+	0.5	12	9	10	14	24	13	42	38	76	81	60	50
	+	1.0	12	9	9	10	22	17	32	29	89	76	49	48
	+	10.0	11	10	8	10	16	11	34	43	96	64	64	54
	+	50.0	10	7	5	12	16	19	35	23	87	79	57	49
	+	100.0	12	9	6	11	19	15	37	23	77	92	41	56
	+	500.0	9	17	8	9	7	17	20	19	65	84	43	42

Table 182

IN VITRO ASSAYS WITH *SACCHAROMYCES CEREVISIAE* D3
p-Dichlorobenzene
Experiment 1

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($x\ 10^{-7}$)	Percent	Per ml ($x\ 10^{-3}$)	Per 10^5 Survivors
Negative control DMSO	-		7.6	100	5.5	7.2
	+		7.3	100	4.5	6.2
Positive control 1,2,3,4-Diepoxybutane	-	0.025	7.5	99	583	780
	+	0.025	6.7	92	593	890
p-Dichlorobenzene	-	0.1	T*		T	
	-	0.5	7.2	95	45	63
	-	1.0	5.5	72	20	36
	-	5.0	5.6	74	3.0	5.4
	+	0.1	4.7	64	21	45
	+	0.5	7.3	100	16	22
	+	1.0	7.5	103	15	20
	+	5.0	5.5	75	12	22

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*T, toxic.

Table 183

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
p-Dichlorobenzene
Experiment 2

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml (x 10^{-7})	Percent	Per ml (x 10^{-3})	Per 10^5 Survivors
Negative control						
DMSO	-		5.7	100	1.0	1.8
	+		6.2	100	3.0	4.8
Positive control						
1,2,3,4-Diepoxybutane	-	0.025	5.7	100	583	1000
	+	0.025	6.2	100	465	750
p-Dichlorobenzene	-	0.1	0.1	2	1.0	100
	-	0.25	1.1	19	4.0	36
	-	0.5	1.3	23	8.0	62
	-	1.0	4.5	79	6.0	13
	-	2.0	6.1	107	4.0	6.6
	-	4.0	4.7	82	2.0	4.3
	+	0.05	0.1	2	0	0
	+	0.5	2.7	44	12.0	44
	+	1.0	3.4	55	5.0	15
	+	2.0	3.9	63	2.0	5.1
	+	4.0	4.5	73	2.0	4.4

Table 184

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
p-Dichlorobenzene
Experiment 3

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml (x 10^{-7})	Percent	Per ml (x 10^{-3})	Per 10^5 Survivors
Negative control						
DMSO	-		4.7	100	4.0	8.5
	+		4.4	100	1.5	3.4
Positive control						
1,2,3,4-Diepoxybutane	-	0.025	4.7	100	423	900
	+	0.025	4.5	102	418	930
p-Dichlorobenzene	-	0.0005	6.3	134	7.0	11
	-	0.001	5.6	119	8.0	14
	-	0.005	6.7	143	1.0	1.5
	-	0.01	4.7	100	7.0	15
	-	0.05	T*			
	+	0.0005	4.7	107	3.0	6.4
	+	0.001	5.4	123	4.0	7.4
	+	0.005	5.1	116	6.0	12
	+	0.01	2.9	66	9.0	31
	+	0.05	T			

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*T, toxic.

Table 185

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
p-Dichlorobenzene
Experiment 4

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml (x 10^{-7})	Percent	Per ml (x 10^{-3})	Per 10^5 Survivors
Negative control DMSO	-		6.1	100	4.5	7.4
	+		6.6	100	2.0	3.0
Positive control 1,2,3,4-Diepoxybutane	-	0.025	5.8	95	373	640
	+	0.025	5.3	80	478	900
p-Dichlorobenzene	-	0.005	7.6	125	2.0	2.6
	-	0.01	7.2	118	2.0	2.8
	-	0.05	T		T	
	-	0.1	T		T	
	-	0.25	T		T	
	-	0.5	T		T	
	-	1.0	T		T	
	-	2.0	0.76	12	1.0	13
	+	0.005	5.6	85	2.0	3.6
	+	0.01	8.4	127	2.0	2.4
	+	0.05	T		T	
	+	0.1	T		T	
	+	0.25	T		T	
	+	0.5	T		T	
	+	1.0	T		T	
	+	2.0	0.6	10	0.0	0.0

Table 186

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
p-Dichlorobenzene
Experiment 5

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors: Cells per ml (x 10^{-7})	Survivors: Percent	Mitotic Recombinants: Per ml (x 10^{-3})	Mitotic Recombinants: Per 10^5 Survivors
Negative control	-		6.2	100	6.0	9.7
	+		7.3	100	6.0	8.2
Positive control 1,2,3,4-Diepoxybutane	-		5.8	94	610	1100
	+		6.0	82	670	1100
p-Dichlorobenzene	-	0.0005	6.6	106	2.0	3.0
	-	0.001	8.8	142	2.0	2.3
	-	0.005	7.1	115	4.0	5.6
	-	0.01	6.6	106	5.0	7.6
	-	0.05	1.5	24	11	73
	-	0.1	T*			
	-	0.25	T		T	
	-	0.5	T		T	
	-	1.0	1.0	16	2.0	20
	-	2.0	0.8	13	2.0	25
	-	4.0	1.3	21	6.0	46
	-	5.0	3.6	58	6.0	17
	+	0.0005	6.3	86	2.0	3.2
	+	0.001	6.3	86	2.0	3.2
	+	0.005	6.2	85	4.0	6.5
	+	0.01	5.7	78	3.0	5.3
	+	0.05	1.1	15	6.0	55
	+	0.1	T		T	
	+	0.25	T		T	
	+	0.5	T		T	
	+	1.0	1.2	16	2.0	17
	+	2.0	2.4	33	12.0	50
	+	4.0	1.3	18	5.0	38
	+	5.0	4.0	55	6.0	15

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*T, toxic.

Table 187

DIFFERENTIAL TOXICITY ASSAYS
p-Dichlorobenzene

		Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		E. coli				B. subtilis			
Compound	Amount	W3110 (polA$^+$)		p3478 (polA$^-$)		H17 (rec$^+$)		M45 (rec$^-$)	
EXPERIMENT 1									
Negative control Ampicillin	0.01 mg	20	20	18	17	25	26	28	27
Positive control Methylmethane sulfonate	1.0	30	30	35	35	14	14	49	46
p-Dichlorobenzene	1.0	6	6	6	6	7	7	7	7
	5.0	6	6	6	6	7	7	7	7
EXPERIMENT 2									
Negative control Ampicillin	0.01 mg	19	18	17	17	25	25	27	27
Positive control Methylmethane sulfonate	1.0	16	16	22	23	15	15	50	50
p-Dichlorobenzene	1.0	6	6	6	6	6	6	6	6
	5.0	6	6	6	6	6	6	6	6

Dinoseb

S. typhimurium Plate Incorporation, E. coli WP-2. Dinoseb was previously tested and found to be negative in both of these systems, with and without activation (51).

S. cerevisiae D3 (Table 188). This compound was previously tested over a narrow dose range and found to be negative both with and without activation (51). It was retested over a wider dose range under the present contract, with similar results.

E. coli/B. subtilis Relative Toxicity. Dinoseb was previously tested and found positive in both species (51).

S. typhimurium Relative Toxicity (Tables 189, 190). This compound was more toxic to the repair-deficient strains than to the repair-proficient derivatives; thus it was positive in this assay.

UDS in WI-38 Cells. Dinoseb was previously tested and found negative in this system, with and without activation (51).

Table 188

IN VITRO ASSAYS WITH *SACCHAROMYCES CEREVISIAE* D3
DINOSEB
Retest

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml (x 10^{-7})	Percent	Per ml (x 10^{-3})	Per 10^5 Survivors
Negative Control: DMSO	-		8.5	100	6.5	7.7
	+		7.7	100	3.0	3.9
Positive Control 1,2,3,4-Diepoxybutane	-	0.025	8.9	105	941.3	1057.6
Dinoseb	-	0.1	3.8	45	3.0	7.9
	-	0.2	5.1	60	5.0	9.8
	-	0.3	5.1	60	4.0	7.8
	-	0.4	2.4	28	3.0	12.5
	-	0.5	T*	T	T	T
	+	0.1	4.7	61	4.0	8.5
	+	0.2	4.6	60	7.0	15.2
	+	0.3	6.0	78	12.0	20.0
	+	0.4	3.0	39	3.0	10.0
	+	0.5	T	T	T	T

---

* T, toxic.

Table 189

DIFFERENTIAL TOXICITY ASSAYS WITH SALMONELLA TYPHIMURIUM STRAINS

Experiment 1

		Zone of Inhibition of Growth (in mm, disc = 6 mm)							
Compound	Amount of Compound Applied to Disc	SL4525 (rec$^+$)		SL4700 (rec$^-$)		TA1978 (uvrB$^+$)		TA1538 (uvrB$^-$)	
Negative Control									
Kanamycin	100.0 µg	14	13	13	14	13	13	14	14
Positive Controls									
MMS[*]	10.0 mg	47	46	68	65				
4-NQO[†]	2.0 mg					12	12	24	23
Dinoseb	1.0 mg	23	22	26	25	21	22	26	25
	5.0 mg	19	20	24	23	20	20	22	22

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[*] MMS = Methylmethanesulfonate.

[†] 4-NQO = 4-Nitroquinoline-1-oxide.

Table 190

DIFFERENTIAL TOXICITY ASSAYS WITH SALMONELLA TYPHIMURIUM STRAINS

Experiment 2

Compound	Amount of Compound Applied to Disc	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		SL4525 (rec^+)		SL4700 (rec^-)		TA1978 ($uvrB^+$)		TA1538 ($uvrB^-$)	
Negative Control									
Kanamycin	100.0 μg	15	14	16	15	17	17	15	16
Positive Controls									
MMS*	10.0 mg	45	40	72	74				
4-NQO†	2.0 mg					12	12	28	26
Dinoseb	1.0 mg	24	25	25	28	13	13	25	26
	5.0 mg	24	22	26	27	17	17	28	29

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* MMS = Methylmethanesulfonate.

† 4-NQO = 4-Nitroquinoline-1-oxide.

Disulfoton

S. typhimurium Plate Incorporation, E. coli WP-2, S. cerevisiae D3. Disulfoton was tested previously and was found to be negative, both with and without activation, in these three test systems (52).

S. cerevisiae D7 (Tables 191, 192). Disulfoton was tested twice over concentrations ranging from 1.0% to 5.0%. When tested up to the concentration limits of the D7 assay, disulfoton exhibited only slight killing; no increase in the three genetic end points was observed.

E. coli/B. subtilis Relative Toxicity. Disulfoton was previously tested in this system and was reported to be negative in both species (52). Reexamination of these results indicates that the tests were inconclusive, rather than negative.

S. typhimurium Relative Toxicity (Tables 193, 194). This compound was negative in both strain pairs in this assay.

SCE in CHO Cells (Tables 195-199). Disulfoton was dissolved in DMSO at a concentration of 50%. At concentrations of 0.1% (in culture medium) and above, with and without metabolic activation, disulfoton caused etching of plastic T-75 tissue culture flasks. Table 195 presents the results of the cytotoxicity test of disulfoton in CHO cell cultures without metabolic activation. At a concentration of 0.02% and above, the cultures yielded primarily M_1 metaphases. At concentrations of 0.004% and less, M_2 metaphases were produced. Table 196 presents the results of the cytotoxicity test of disulfoton in CHO cell cultures with metabolic activation. At concentrations of 0.1% and above, primarily M_1 metaphases were produced; and at concentrations of 0.02% and less, primarily M_2 metaphases were produced.

Table 197 presents the SCE frequencies in CHO cells exposed to disulfoton without metabolic activation. A one-way analysis of variance was conducted on SCE frequencies of the five concentrations of disulfoton and of the negative controls. The variance between treatment groups was not significantly greater than the variance within treatment groups. Table 198 presents the SCE frequencies in CHO cells exposed to disulfoton with metabolic activation. The variance between treatment groups was not significantly greater than the variance within treatment groups, but there appeared to be a concentration-related response.

A third cytogeneticist was asked to evaluate these coded slides. Table 199 presents the observations of the three cytogeneticists. Like A and B, cytogeneticist C's observations also indicated a concentration-related increase in SCE frequencies. Analysis of the results revealed that the variance between treatment groups was significantly greater than the variance within treatment groups. We interpret these results to indicate that with metabolic activation, disulfoton caused a concentration-related increase in the SCE frequencies of CHO cells.

The test of disulfoton with metabolic activation involved exposing CHO cells to a higher concentration range for a shorter time (0.0025% to 0.04% for 2 hours) than that used in the test without metabolic activation (0.0005% to 0.008% for 21.5 hours). Therefore, direct comparisons are inappropriate.

However, we conclude that disulfoton does require metabolic activation to increase the SCE frequencies in CHO cells.

L5178Y Mouse Lymphoma (Tables 200-203). Concentration-range testing of disulfoton in the absence of the metabolic activation system (Table 200) indicated that the range to use in the mutagenesis test was approximately 1 to 80 μg/ml (98.3% relative total growth at 1 μg/ml and 9.0% relative total growth at 80 μg/ml). Concentration-range test results of disulfoton in the presence of the metabolic activation system (Table 201) showed that the approximate range to use in the mutagenesis assay was 20 to 100 μg/ml (126.1% relative total growth at 20 μg/ml and 13.9% relative total growth at 100 μg/ml).

Table 202 presents the results of mutagenesis testing of disulfoton in the absence of the metabolic activation system. The highest concentration tested, 90 μg/ml, yielded relative total growth of 5.3%. No significant depression in relative total growth was observed at a concentration of 15 μg/ml or less. At 15 μg/ml the average relative total growth was 97.0%. Over the range tested, a concentration-related increase in induced mutation frequency was observed. A mutation frequency at least twice the spontaneous frequency was observed at 70 μg/ml with a relative total growth value of 12.4%. For the present, we interpret these results as positive, but this test should be repeated in order to confirm this conclusion.

Table 203 presents the results of mutagenesis testing of disulfoton in the presence of the metabolic activation system. Disulfoton was extremely toxic at concentrations greater than 102 μg/ml, yielding relative suspension growth values of less than 10.0%. The lowest concentration tested, 27 μg/ml, yielded an average relative total growth value of 68.5%. Over the range tested there was some evidence of a concentration-related increase in induced mutation frequency, but none of the concentrations showed a mutation frequency at least twice the spontaneous frequency.

These combined results indicate a mutagenic effect of disulfoton at the thymidine kinase locus in L5178Y mouse lymphoma cells in the absence of the metabolic activation system, but not in the presence of the metabolic activation system. In the absence of the metabolic activation system, disulfoton appeared to increase the mutation frequency, in a concentration-related manner, above the frequencies observed with the solvent control. This result was not observed in the presence of the metabolic activation system.

UDS in WI-38 Cells. Disulfoton was previously tested and found to be positive without activation, negative with activation (52).

Micronucleus Test (Tables 204, 205). The dosing and sacrifice schedules are shown in Table 204. The incidences of micronucleated PCEs in blood and bone marrow and the ratios of PCEs to mature red cells (RBCs) are given in Table 205. The results for all vehicle control and disulfoton-treated groups were negative ($\beta < 0.05$); the positive control (TMP-treated) groups were positive ($\alpha < 0.001$). The mean ratio of PCEs to RBCs was within the historical control range (0.5-1.5) for all dose-time groups.

The values obtained for the blood smears were comparable to the results for the bone marrow for the negative-control, compound-treated, and 72-hour positive-control groups. The result for the 30-hour TMP treated groups was positive, although markedly less so than the bone-marrow results for the same animals. The negative result in the 48-hour TMP blood-smear group was thought to be a chance anomaly.

The dose-related weight loss observed 24 hours after the first dose, along with the fatalities in the high-dose group, indicates that a maximum tolerated dose was administered. In this micronucleus test, a sufficient number of cells and time points were examined at each dose level, the maximum possible dose was given, and the PCE-RBC ratio was not depressed. The results for all treatment groups were negative. Disulfoton is therefore negative in the micronucleus test.

Table 191

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D7

DISULFOTON

Experiment 1

			Survivors		Crossing-Over				Gene Conversion		Reverse Mutation	
					Mitotic Recombinants		Total Aberrants		Convertants		Revertants	
Compound	MA	% Conc.	Cells Per ml ($\times 10^{-7}$)	%	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors	Per ml ($\times 10^{-1}$)	Per 10^6 Survivors	Per ml ($\times 10^0$)	Per 10^7 Survivors
Negative Control												
DMSO	-		5.0	100	1.0	2.0	19	38	53	11	8.0	0.8
	+		5.5	100	1.0	1.8	19	35	72	13	6.0	1.1
Positive Controls												
1,2,3,4-Diepoxybutane	-	0.006	4.5	90	70	160	800	1,800	1,300	290	270	60
	+	0.006	6.6	120	63	95	690	1,000	800	120	100	15
Disulfoton	-	1.0	4.9	98	6.0	12	36	73	61	12	11	2.2
	-	2.0	4.6	92	0	0	13	28	62	13	13	2.8
	-	3.0	5.6	110	3.0	5.4	16	29	55	9.8	25	4.5
	-	4.0	5.5	110	2.0	3.6	13	24	25	4.5	3.0	0.5
	-	5.0	5.2	100	1.0	1.9	17	33	86	17	18	3.5
	+	1.0	4.7	85	0	0	9.0	19	98	21	17	3.6
	+	2.0	5.1	93	0	0	23	45	74	15	2.0	0.4
	+	3.0	4.9	89	4.0	8.2	33	67	87	18	15	3.1
	+	4.0	5.6	100	1.0	1.8	40	71	110	20	4.0	0.7
	+	5.0	4.9	89	1.0	2.0	15	31	36	7.3	0	0

Table 192

IN VITRO ASSAYS WITH *SACCHAROMYCES CEREVISIAE* D7

DISULFOTON

Experiment 2

			Survivors		Crossing-Over				Gene Conversion		Reverse Mutation	
			Cells		Mitotic Recombinants		Total Aberrants		Convertants		Revertants	
Compound	MA	% Conc.	Per ml ($\times 10^{-7}$)	%	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors	Per ml ($\times 10^{-3}$	Per 10^5 Survivors	Per ml ($\times 10^{-1}$)	Per 10^5 Survivors	Per ml ($\times 10^{0}$)	Per 10^5 Survivors
Negative Control												
DMSO	-		6.0	100	0.5	0.8	9.0	15	58	9.7	44	7.3
	+		6.0	100	0.5	0.8	8.0	13	73	12	40	6.7
Positive Controls												
1,2,3,4-Diepoxybutane	-	0.006	5.9	98	78	130	180	300	460	78	140	24
	+	0.006	5.9	98	73	120	140	240	340	58	110	19
Disulfoton	-	1.0	6.2	100	0	0	16	26	73	12	38	6.1
	-	2.0	5.4	90	1.0	1.9	19	35	45	8.3	59	11
	-	3.0	4.9	82	0	0	6.0	12	67	14	29	5.9
	-	4.0	5.6	93	0	0	9.0	16	77	14	73	13
	-	5.0	5.1	85	1.0	2.0	16	31	79	15	66	13
	+	1.0	4.7	78	1.0	2.1	7.0	15	77	16	69	15
	+	2.0	5.1	85	0	0	7.0	14	70	14	55	11
	+	3.0	5.7	95	0	0	10	18	73	13	82	14
	+	4.0	5.2	87	0	0	4.0	7.7	60	12	80	15
	+	5.0	5.1	85	0	0	13	25	84	16	77	15

Table 193

DIFFERENTIAL TOXICITY ASSAYS WITH *SALMONELLA* *TYPHIMURIUM* STRAINS

Experiment 1

Compound	Amount of Compound Applied to Disc	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		SL4525 (rec$^+$)		SL4700 (rec$^-$)		TA1978 (uvrB$^+$)		TA1538 (uvrB$^-$)	
Negative Control									
Kanamycin	100.0 µg	14	13	13	14	13	13	14	14
Positive Controls									
MMS[*]	10.0 mg	47	46	68	65				
4-NQO[†]	2.0 mg					12	12	24	23
Disulfoton	5.0 µl	6	6	6	6	6	6	6	6
	10.0 µl	6	6	6	6	6	6	6	6

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[*] MMS = Methylmethanesulfonate.

[†] 4-MQO = 4-Nitroquinoline-1-oxide.

Table 194

DIFFERENTIAL TOXICITY ASSAYS WITH SALMONELLA TYPHIMURIUM STRAINS

Experiment 2

Compound	Amount of Compound Applied to Disc	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		SL4525 (rec$^+$)		SL4700 (rec$^-$)		TA1978 (uvrB$^+$)		TA1538 (uvrB$^-$)	
Negative Control									
Kanamycin	100.0 µg	15	14	16	15	17	17	15	16
Positive Controls									
MMS*	10.0 mg	45	40	72	74				
4-NQO†	2.0 mg					12	12	28	26
Disulfoton	10.0 µl	6	6	6	6	6	6	6	6
	20.0 µl	7	7	8	8	7	7	7	7

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* MMS = Methylmethanesulfonate.

† 4-NQO = 4-Nitroquinoline-1-oxide.

Table 195

CYTOTOXICITY TEST OF CHINESE HAMSTER OVARY CELLS EXPOSED TO DISULFOTON FOR 21.5 HOURS WITHOUT METABOLIC ACTIVATION

Treatment	Number of Divisions in Bromodeoxyuridine (BrdU)[*]
Negative control (1% DMSO)	M_2
Disulfoton	
0.0008	M_2
0.004	M_2
0.02	M_1
0.1[†]	M_1
0.5[†]	M_1
Positive control (10^{-3} M ethylmethanesulfonate)	M_2

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[*] M_1, one division in BrdU M_2, two divisions in BrdU.

[†] Etching of plastic T-75 tissue culture flasks observed.

Table 196

CYTOTOXICITY TEST OF CHINESE HAMSTER OVARY CELLS
EXPOSED TO DISULFOTON FOR 2 HOURS
WITH METABOLIC ACTIVATION

Treatment	Number of Divisions in Bromodeoxyuridine (BrdU)[*]
Negative control (1% DMSO)	M_2
Disulfoton (%)	
0.0008	M_2
0.004	M_2
0.02	M_2
0.1[‡]	M_1
0.5[‡]	$M_1 > M_2$[†]
Positive control (10^{-3} M dimethylnitrosamine)	M_2

---

[*] M_1, one division in BrdU; M_2, two divisions in BrdU.

[†] The cell type to the left of the > sign is in excess.

[‡] Etching of plastic T-75 tissue culture flasks observed.

Table 197

SISTER CHROMATID EXCHANGE (SCE) FREQUENCIES IN CHINESE HAMSTER OVARY (CHO) CELLS
EXPOSED TO DESULFOTON FOR 21.5 HOURS WITHOUT METABOLIC ACTIVATION

Treatment	Cyto-geneticist*	No. of SCEs	No. of Chromosomes	Chromosomes/Cell (Mean ± SD)	SCEs/Cell† (Mean ± SE)	SCEs/Chromosome† (Mean ± SE)
Negative control (1% DMSO)	A	316	484	19.36±0.64	12.64±0.71	0.653±0.037
	B	252	496	19.84±0.75	10.08±0.63	0.508±0.032
Disulfoton (%)						
0.0005	A	245	486	19.44±0.65	9.80±0.63	0.504±0.032
	B	272	495	19.80±0.76	10.88±0.66	0.549±0.033
0.001	A	296	487	19.48±0.82	11.84±0.69	0.608±0.035
	B	272	497	19.88±0.60	11.12±0.67	0.559±0.034
0.002	A	297	488	19.52±0.59	11.88±0.69	0.609±0.035
	B	265	493	19.72±0.46	10.60±0.65	0.538±0.033
0.004	A	277	479	19.16±1.07	11.08±0.67	0.578±0.035
	B	370	503	20.12±0.78	14.80±0.77	0.736±0.038
0.008	A	327	475	19.00±0.58	13.08±0.72	0.688±0.038
	B	343	499	19.96±0.45	13.72±0.74	0.687±0.037
Positive control (10^{-3} M ethyl methanesulfonate)	A	949	475	19.00±0.65	37.96±1.23	1.998±0.065
	B	971	497	19.88±0.73	38.84±1.25	1.954±0.063

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[*] Each cytogeneticist analyzed 25 cells per sample.

[†] A one-way analysis of variance comparing the SCE frequencies in CHO cells exposed to disulfoton and to the negative control indicated that the variance between treatment groups was not significantly greater than the variance within treatment groups.

Table 198

SISTER CHROMATID EXCHANGE (SCE) FREQUENCIES IN CHINESE HAMSTER OVARY (CHO) CELLS EXPOSED TO DISULFOTON FOR 2 HOURS WITH METABOLIC ACTIVATION

Treatment	Cyto-geneticist*	No. of SCEs	No. of Chromosomes	Chromosomes/Cell (Mean ± SD)	SCEs/Cell (Mean ± SE)	SCEs/Chromosome† (Mean ± SE)
Negative Control (1% DMSO)	A	297	495	19.80±0.76	11.88±0.69	0.600±0.035
	B	302	494	19.76±0.72	12.08±0.70	0.611±0.035
Disulfoton (%)						
0.0025	A	310	485	19.40±0.96	12.40±0.70	0.639±0.036
	B	323	495	19.64±0.64	12.92±0.72	0.658±0.037
0.005	A	258	504	20.16±0.55	10.32±0.64	0.512±0.032
	B	337	495	19.80±0.91	13.48±0.73	0.681±0.037
0.01	A	278	489	19.56±0.65	11.12±0.67	0.569±0.034
	B	301	483	19.32±0.75	12.04±0.69	0.623±0.036
0.02	A	310	492	19.68±0.56	12.40±0.70	0.630±0.036
	B	384	477	19.08±0.70	15.36±0.78	0.805±0.041
0.04	A	400	486	19.44±0.77	16.00±0.80	0.823±0.041
	B	410	485	19.40±0.96	16.40±0.81	0.845±0.042
Positive control (10^{-3} M dimethylnitrosamine)	A	779	499	19.96±0.68	31.16±1.12	1.561±0.056
	B	769	495	19.80±1.08	30.76±1.11	1.554±0.056

---

[*] Each cytogeneticist analyzed 25 cells per sample.

[†] A one-way analysis of variance comparing the SCE frequencies in CHO cells exposed to disulfoton and to the negative control indicated that the variance between treatment groups was not significantly greater than the variance within treatment groups.

Table 199

SISTER CHROMATID EXCHANGE (SCE) FREQUENCIES IN CHINESE HAMSTER OVARY (CHO) CELLS EXPOSED TO DISULFOTON FOR 2 HOURS WITH METABOLIC ACTIVATION

Treatment	Sample Number	Cyto-geneticist*	No. of SCEs	No. of Chromosomes	Chromosomes/Cell (Mean ± SD)	SCEs/Cell (Mean ± SE)	SCEs/Chromosome† (Mean ± SD)
Negative Control (1% DMSO)	1	A	297	495	19.80±0.76	11.88±0.69	0.600±0.035
	2	B	302	494	19.76±0.72	12.08±0.70	0.611±0.035
	2	C	321	482	19.28±1.10	12.84±0.72	[illegible]66±0.037
Disulfoton (%)							
0.0025	3	A	310	485	19.40±0.96	12.40±0.70	0.639±0.036
	4	B	323	495	19.64±0.64	12.92±0.72	0.658±0.037
0.005	5	B	337	495	19.80±0.91	13.48±0.73	0.681±0.037
	5	C	330	482	19.28±0.79	13.20±0.73	0.685±0.038
	6	A	258	504	20.16±0.55	10.32±0.64	0.512±0.032
	6	C	258	476	19.04±0.89	10.32±0.64	0.542±0.034
0.01	7	A	278	489	19.56±0.65	11.12±0.67	0.569±0.034
	8	B	301	483	19.32±0.75	12.04±0.69	0.623±0.036
0.02	9	B	384	477	19.08±0.70	15.36±0.78	0.805±0.041
	9	C	349	473	18.92±1.04	13.96±0.75	0.738±0.039
	10	A	310	492	19.68±0.56	12.40±0.70	0.630±0.036
	10	C	256	471	18.84±0.85	10.24±0.64	0.544±0.034
0.04	11	B	410	485	19.40±0.96	16.40±0.81	0.845±0.042
	11	C	487	484	19.36±0.76	19.48±0.88	1.006±0.046
	12	A	400	486	19.44±0.77	16.00±0.80	0.823±0.041
Positive control (10^{-3} M dimethylnitrosamine)	13	B	769	495	19.80±1.08	30.76±1.11	1.554±0.056
	14	A	779	499	19.96±0.68	31.16±1.12	1.561±0.056

---

[*] Each cytogeneticist analyzed 25 cells per sample.

[†] A one-way analysis of variance comparing the SCE frequencies in CHO cells exposed to disulfoton and to the negative control indicated that the variance between treatment groups was significantly greater ($p=0.01$) than the variance within treatment groups.

Table 200

MOUSE LYMPHOMA FORWARD MUTATION CONCENTRATION-RANGE ASSAY OF DISULFOTON IN THE ABSENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency (× 10⁻⁶)	Induced Mutation Frequency (× 10⁻⁶)
Solvent control (1% DMSO)	104.4 95.6	600 600	725 704	129 188	101.5 98.5	105.9 94.2	36 53	-- --
Positive control (500 μg/ml EMS)	64.3 64.5	600 600	381 337	1310 --*	53.3 47.2	34.3 30.4	688 --	643 --
Disulfoton								
1	104.1	600	675	179	94.5	98.3	53	8
2	99.7	600	667	167	93.4	93.1	50	5
5	104.4	600	564	183	78.9	82.4	65	20
10	104.4	600	641	155	89.7	93.7	48	3
20	99.5	600	565	--*	79.1	78.7	--	--
40	58.4	600	584	223	81.7	47.7	76	31
80	12.9	600	500	227	70.0	9.0	91	46
100	2.4	NC†	--	--	--	--	--	--
150	1.3	NC	--	--	--	--	--	--
200	1.3	NC	--	--	--	--	--	--
300	PPT‡	--	--	--	--	--	--	--
400	PPT	--	--	--	--	--	--	--
500	PPT	--	--	--	--	--	--	--
600	PPT	--	--	--	--	--	--	--
700	PPT	--	--	--	--	--	--	--
800	PPT	--	--	--	--	--	--	--
900	PPT	--	--	--	--	--	--	--
1000	PPT	--	--	--	--	--	--	--

---

*Sample lost
†Not cloned
‡Persistent precipitate present after washdown procedure; sample discarded

Table 201

MOUSE LYMPHOMA FORWARD MUTATION CONCENTRATION-RANGE ASSAY OF DISULFOTON IN THE PRESENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency (× 10^{-6})	Induced Mutation Frequency (× 10^{-6})
Solvent control	100.0	600	578	564	100.0	100.0	195	--
(1% DMSO)	--*	--	--	--	--	--	--	--
Positive control	54.9	600	315	988	54.5	29.9	627	432
(5 μg/ml 3-MCA)	51.2	600	385	1092	66.6	34.1	567	372
Disulfoton								
1	106.8	600	649	537	112.3	119.9	165	--
2	107.4	600	560	542	96.9	104.1	194	--
5	117.4	600	700	536	121.1	142.2	153	--
10	127.3	600	632	495	109.3	139.2	157	--
20	101.8	600	716	446	123.9	126.1	125	--
40	44.2	600	657	478	113.7	50.2	146	--
80	15.7	600	637	478	110.2	17.3	150	--
100	13.1	600	613	537	106.1	13.9	175	
150	8.6	600	--*	411	--	--	--	--
200	7.6	NC†	--	--	--	--	--	--
300	3.6	NC	--	--	--	--	--	--
400	0.9	NC	--	--	--	--	--	--
500	1.0	NC	--	--	--	--	--	--
600	1.1	NC	--	--	--	--	--	--
700	1.0	NC	--	--	--	--	--	--
800	1.1	NC	--	--	--	--	--	--
900	0.4	NC	--	--	--	--	--	--
1000	1.0	NC	--	--	--	--	--	--

---

*Sample lost
†Not cloned

Table 202

MOUSE LYMPHOMA FORWARD MUTATION ASSAY OF DISULFOTON IN THE ABSENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency ($\times 10^{-6}$)	Induced Mutation Frequency ($\times 10^{-6}$)
Solvent control (1% DMSO)	99.9	600	570	87	101.5	101.4	31	--
	100.1	600	553	120	98.5	98.6	43	--
Positive control (500 μg/ml EMS)	64.1	600	316	1241	56.3	36.1	785	748
	66.5	600	341	1211	60.7	40.4	710	673
Disulfoton								
5	104.7	600	525	89	93.5	97.9	34	--
	109.6	600	591	96	105.3	115.4	32	--
15	91.5	600	576	122	102.6	93.9	42	5
	91.7	600	613	147	109.2	100.1	48	11
30	67.3	600	572	128	101.9	68.6	45	8
	77.8	600	502	113	89.4	69.6	45	8
40	48.9	600	--*	145	--	--	--	--
	50.4	600	603	149	107.4	54.1	49	12
50	43.8	600	544	168	96.9	42.4	62	25
	53.2	600	520	168	92.6	49.3	65	28
60	12.2	600	--*	183	--	--	--	--
	3.5	NC†	--	--	--	--	--	--
70	13.1	600	533	215	94.9	12.4	81	44
	15.7	600	530	--	94.4	14.8	--	--
75	9.0	600	--	326	--	--	--	--
	9.5	600	527	174	93.9	8.9	66	29
80	11.2	600	463	219	82.5	9.2	95	58
	14.7	600	521	--*	92.8	13.6	--	--
90	2.2	NC†	--	--	--	--	--	--
	7.7	600	388	203	69.1	5.3	105	68

---

*Sample lost.

†Not cloned.

Table 203

MOUSE LYMPHOMA FORWARD MUTATION ASSAY OF DISULFOTON IN THE PRESENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency (× 10⁻⁶)	Induced Mutation Frequency (× 10⁻⁶)
Solvent control (1% DMSO)	96.0	600	560	280	103.1	99.0	100	--
	104.0	600	526	272	96.9	100.7	103	--
Positive control (6 μg/ml 3-MCA)	31.7	600	263	483	48.4	15.4	367	265
	33.6	600	208	524	38.3	12.9	504	402
Disulfoton								
27	64.1	600	567	154	104.4	66.9	54	--
	65.3	600	583	196	107.4	70.1	67	--
36	48.4	600	494	199	91.0	44.0	81	--
	41.2	600	464	204	85.5	35.2	88	--
45	30.2	600	493	234	90.8	27.4	95	--
	37.4	600	427	222	78.6	29.4	104	2
60	27.6	600	417	213	76.8	21.2	102	--
	25.6	600	458	226	84.3	21.6	99	--
75	22.7	600	513	225	94.5	21.4	88	--
	32.9	600	494	178	91.0	29.9	72	--
90	14.2	600	480	256	88.4	12.6	107	5
	15.6	600	483	215	89.0	13.9	89	--
102	12.3	600	517	391	95.2	11.7	151	49
	14.5	600	435	271	80.1	11.6	125	23
126	7.2	NC*	--	--	--	--	--	--
	9.3	NC	--	--	--	--	--	--
135	6.1	NC	--	--	--	--	--	--
	8.0	NC	--	--	--	--	--	--
150	5.6	NC	--	--	--	--	--	--
	8.0	NC	--	--	--	--	--	--

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*Not cloned.

Table 204

DOSAGE AND SACRIFICE SCHEDULE

Compound	Animals	Dose at 0 and 24 Hr	Mean Body Weight Change (g)	No. Sacrificed			No. Fatalities
				30 Hr	48 Hr	72 Hr	
TMP--Positive control	12	1.25 g/kg	-1.6 ± .9	4	4	4	0
DMSO--Vehicle control	24	5 ml/kg	-0.7 ± 0.9	8	8	8	0
Disulfoton							
Low Dose	24	2 mg/kg	-1.1 ± .8	8	8	8	0
Medium Dose	24	4 mg/kg	-1.3 ± .8	8	8	8	0
High Dose	24	8 mg/kg	-1.6 ± .8	8	7	7	2

Table 205

INCIDENCE OF MICRONUCLEATED POLYCHROMATOPHILIC ERYTHROCYTES IN BLOOD AND BONE MARROW*

(500 PCEs Scored per Smear)

	30 Hours				48 Hours				72 Hours			
Dose	Animal No.	Blood PCE with MN	BM PCE with MN	BM PCE/RBC	Animal No.	Blood PCE with MN	BM PCE with MN	BM PCE/RBC	Animal No.	Blood PCE with MN	BM PCE with MN	BM PCE/RBC
DMSO 5 ml/kg	C0	+	0	1.3	A0	1	0	0.7	B0	1	1	0.9
	C1	+	0	1.0	A1	0	0	1.3	B1	1	0	1.2
	C2	+	1	1.0	A2	0	1	0.9	B2	0	1	0.6
	C3	+	1	0.7	A3	0	0	1.4	B3	-	1	0.9
	D0	+	2	1.0	E0	0	0	0.9	F0	0	1	2.7
	D1	+	0	1.3	E1	1	0	1.0	F1	0	1	1.2
	D2	+	1	1.0	E2	0	0	0.9	F2	0	0	2.8
	D3	+	3	1.2	E3	1	0	0.7	F3	0	2	0.9
	Total		8			3	1			2	8	
	Mean			1.1±.1				1.0±.3				1.4±.9
TMP 1.25 g/kg	J0	2	30	0.6	H0	4	21	0.7	I0	6	8	0.5
	J1	3	10	0.3	H1	0	13	0.6	I1	2	0	0.8
	J2	6	8	0.6	H2	0	20	1.3	I2	3	3	0.3
	J3	+	20	0.4	H3	0	3	0.2	I3	1	0	0.7
	Total	11	68			4	57			12	11	
	Mean			0.5±.15				0.7±.5				0.6±.2
Disulfoton 2 mg/kg	XB0	1	0	1.8	XC0	1	0	0.6	XA0	0	1	0.7
	XB1	1	1	1.4	XC1	2	0	0.6	XA1	1	1	0.6
	XB2	1	0	1.5	XC2	0	0	1.0	XA2	0	0	0.9
	XB3	0	0	1.0	XC3	0	0	0.8	XA3	0	0	1.1
	XF0	0	1	1.0	XD0	1	0	1.2	XE0	0	0	1.1
	XF1	0	1	1.3	XD1	0	0	1.2	XE1	1	0	2.2
	XF2	0	1	1.1	XD2	0	1	0.9	XE2	0	1	2.2
	XF3	0	1	1.0	XD3	2	2	0.8	XE3	0	1	0.7
	Total	3	5			5	3			2	4	
	Mean			1.3±.3				0.9±.2				1.2±.7
Disulfoton 4 mg/kg	XG0	1	2	0.8	XH0	1	0	0.4	XI0	0	0	1.0
	XG1	0	2	0.6	XH1	0	1	0.6	XI1	0	1	1.1
	XG2	0	0	0.8	XH2	2	0	0.8	XI2	0	0	0.6
	XG3	1	0	1.4	XH3	0	0	0.7	XI3	0	0	1.0
	XJ0	1	1	0.8	XK0	0	0	1.8	XL0	+	1	0.8
	XJ1	0	1	1.1	XK1	0	1	1.8	XL1	+	0	1.2
	XJ2	0	0	0.6	XK2	0	0	2.2	XL2	0	0	1.7
	XJ3	1	1	1.1	XK3	0	1	2.9	XL3	0	0	1.2
	Total	4	7			3	3			0	2	
	Mean			0.9±.3				1.4±.9				1.1±.3
Disulfoton 8 mg/kg	XP0	0	1	1.0	XM1	0	0	1.7	XN0	0	1	1.1
	XP1	2	1	1.6	XM2	0	0	0.7	XN1	1	0	0.5
	XP2	1	0	2.2	XM3	0	0	1.1	XN2	0	1	0.9
	XP3	2	0	0.7	XQ0	1	0	1.2	XN3	0	2	0.8
	XR0	0	2	1.0	XQ1	0	0	0.8	XO0	0	1	1.1
	XR1	1	0	0.8	XQ2	0	0	1.8	XO1	0	1	1.1
	XR2	0	0	1.1	XQ3	3	0	0.7	XO2	0	0	0.9
	XR3	0	0	0.6								
	Total	6	4			4	0			1	6	
	Mean			1.1±.5				1.1±.5				0.9±.2

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* BM = bone marrow; PCE = polychromatophilic erythrocytes; RBC = mature erythrocyte; MN = micronucleus.
+ Blood smears for these animals were not evaluated.

DSMA

S. typhimurium Plate Incorporation (Table 206). Previous testing indicated that DSMA was negative in strains TA100, TA1535, TA1537, and TA1538, with and without activation (51). To complete the standard five-strain assay it was tested in strains TA98 and TA100 under the present contract and was found to be negative in both strains with and without activation.

E. coli WP-2. DSMA was found to be negative with and without activation in previous tests (51).

S. cerevisiae D3 (Table 207). This compound was previously tested over a narrow dose range and found to be negative with and without activation (51). It was retested over a wider dose range under the present contract with similar results.

E. coli/B. subtilis Relative Toxicity, UDS in WI-38 Cells. DSMA was previously tested in these systems and was found to be negative for relative toxicity and negative both with and without activation in the UDS system (51).

Table 206

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

DSMA
Retest

Compound	Metabolic Activation	Compound Added (µg/plate)	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative Control							
DMSO	-				25 16	28 30	135 138
	+				23 25	28 38	139 132
Positive Controls:							
2-Anthramine	-	1.0			52	42	145
	+	1.0			310	324	746
Sodium Azide	-	0.5					397
2-Nitrofluorene	-	5.0			817	543	
DSMA	-	500.0				16	116
	-	1,000.0				18	117
	-	2,000.0				24	124
	-	3,000.0				21	131
	-	4,000.0				26	146
	-	5,000.0				27	147
	+	500.0				30	119
	+	1,000.0				32	112
	+	2,000.0				28	109
	+	3,000.0				39	118
	+	4,000.0				35	116
	+	5,000.0				30	124

Table 207

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

DMSA

Retest

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors: Cells per ml ($x\ 10^{-7}$)	Survivors: Percent	Mitotic Recombinants: Per ml ($x\ 10^{-3}$)	Mitotic Recombinants: Per 10^5 Survivors
Negative Control: DMSO	-		8.5	100	6.5	7.7
	+		7.7	100	3.0	3.9
Positive Control 1,2,3,4-Diepoxybutane	-	0.025	8.9	105	941.3	1057.6
DSMA	-	1	11.2	132	3.8	3.4
	-	2	7.8	92	1.3	1.7
	-	3	5.3	62	3.8	7.2
	-	4	6.5	76	2.0	3.1
	-	5	7.6	89	5.0	6.6
	+	1	8.7	113	3.8	4.8
	+	2	13.4	174	6.3	4.7
	+	3	6.9	90	6.0	8.7
	+	4	8.2	106	4.0	4.9
	+	5	6.1	79	3.0	4.9

Folpet

S. typhimurium Plate Incorporation (Table 208). Folpet had been previously tested in strains TA100, TA1535, TA1537 and TA1538 (51). It was found to be positive in the former two strains and negative in the latter two, both with and without activation. Since the data included unusually low values and were variable, it was retested under the present contract in strains TA1537 and TA1538. It was found to be positive in TA1537, but negative in TA1538, both with and without activation. Thus, folpet is positive in this test system.

E. coli WP-2. Folpet was previously tested and found to be positive with and without activation (51).

S. cerevisiae D3 (Table 209). This compound was positive, with and without activation in this system in a previous test at one dose level (51) and also in the retest at several dose levels under the present contract.

E. coli/ B. subtilis Relative Toxicity. Folpet was positive in both species in a previous test (51).

S. typhimurium Relative Toxicity (Tables 210, 211). Folpet was reproducibly more toxic to the repair-deficient strains than to the repair-proficient derivatives. Thus, it was positive in this test system.

L5178Y Mouse Lymphoma (Tables 212-215). In the initial concentration-range assay with folpet, in the absence of the metabolic activation system, extreme cytotoxicity was observed at all the tested concentrations, with relative suspension growth never rising above 0.3%. Therefore, a second concentration-range assay was performed with 18 concentrations between 0.01 and 1 µg/ml (Table 212). The results of the second assay showed that the approximate range to use in the mutagenesis assay was between 0.05 and 0.4 µg/ml, based on relative total growth values of 103.1% and 11.2%, respectively.

Table 213 presents the results of concentration-range testing of folpet in the presence of the metabolic activation system. The results showed that the approximate range to use in the mutagenesis assay was between 5 and 20 µg/ml, based on relative total growth values of 100.6% and 21.2%, respectively.

Table 214 presents the results of mutagenesis testing of folpet in the absence of metabolic activation. The highest concentration of 0.6 µg/ml yielded an average relative total growth value of 6.5%. No significant depression in the relative total growth value was observed at the lowest concentration tested of 0.03 µg/ml, which yielded an average realtive total growth value of 98.6%. From 0.03 to 0.60 µg/ml, a concentration-related increased in induced mutation frequency was observed. Mutation frequencies of at least twice the spontaneous frequency were observed from 0.30 to 0.51 µg/ml (five concentrations), with relative total growth values over 10.0%.

Table 215 presents the results of mutagenesis testing of folpet in the presence of the metabolic activation system. The highest concentration tested (30 µg/ml), yielded an average relative total growth value of 1.4%. No significant depression in the relative total growth was observed at 5 µg/ml, which yielded an average relative total value of 96.2%. From 5 to 30 µg/ml, a concentration-related increase in induced mutation frequency was observed. Average mutation frequencies of at least twice the spontaneous frequency were observed from 20 to 25 µg/ml (four concentrations), with average relative total growth values of over 10%. Although the mutation frequencies of the solvent controls exceeded the stated acceptable levels, this testing was not repeated because: (a) a complete range from cytotoxicity to survival was observed over the tested concentrations of folpet, and (b) the mutation frequencies of the tested samples ranged from the solvent control level to several times the spontaneous mutation frequency, with relative total growth values of over 10%. It may be that our stated criteria for acceptable solvent control levels is too stringent. The National Cancer Institute, in reviewing the data from over 200 experiments performed to validate the L5178Y mutagenesis assay system, has found that spontaneous (solvent control) mutation frequencies of as high as 150×10^{-6} are acceptable if other criteria are met and the test results are positive (57).

The results from these assays indicate a mutagenic effect of folpet at the thymidine kinase locus in L5178Y mouse lymphoma cells, both with and without the metabolic activation system. In both cases, folpet increased the mutation frequencies, in a concentration-related manner, above the spontaneous frequencies observed in the controls. The presence of the metabolic activation system was not required to elicit the mutagenic effect, but it appeared to decrease the cytotoxicity of folpet.

UDS in WI-38 Cells (Tables 216, 217). In a previous test, this compound was reported as negative without and positive with activation (51). Because the control values for this previous test were somewhat lower than usual, it was retested under the present contract. It was found to be negative, both with and without activation.

Mouse Dominant Lethal Test. Folpet was previously tested in this system and found negative (51).

Table 208

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

FOLPET
Retest

Compound	Metabolic Activation	Compound Added (µg/plate)	Histidine Revertants per Plate TA1535	TA1537	TA1538	TA98	TA100
Negative Control							
DMSO	-			7 11	13 17		
	+			7 7	28 15		
Positive Controls							
2-Anthramine	-	1.0			15		
	+	1.0			947		
	-	2.5		19			
	+	2.5		88			
9-Aminoacridine	-	50.0		256			
Sodium Azide	-	0.5					
2-Nitrofluorene	-	5.0			816		
Folpet	-	0.5		5	9		
	-	1.0		7	10		
	-	5.0		19	5		
	-	10.0		67	19		
	-	50.0		311	41		
	-	100.0		215	35		
	-	500.0		T*	T		
	+	0.5		9	26		
	+	1.0		6	15		
	+	5.0		15	15		
	+	10.0		32	33		
	+	50.0		286	45		
	+	100.0		280	55		
	+	500.0		T	T		

---

*T = toxic.

Table 209

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

FOLPET

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($x\ 10^{-7}$)	Percent	Per ml ($x\ 10^{-3}$)	Per 10^5 Survivors
Negative Control: DMSO	-		8.5	100	6.5	7.7
	+		7.7	100	3.0	3.9
Positive Control 1,2,3,4-Diepoxybutane	-	0.025	8.9	105	941.3	1057.6
Folpet	-	0.001	6.9	81	30	43.5
	-	0.002	8.5	100	63	74.1
	-	0.003	7.4	87	43	58.1
	-	0.004	5.9	69	64	108.5
	-	0.005	4.1	48	54	131.7
	+	0.001	9.2	119	23	25.0
	+	0.002	9.8	127	67	68.4
	+	0.003	9.2	119	50	54.4
	+	0.004	9.4	122	44	46.8
	+	0.005	7.4	96	15	20.3

Table 210

DIFFERENTIAL TOXICITY ASSAYS WITH SALMONELLA TYPHIMURIUM STRAINS

Experiment 1

Compound	Amount of Compound Applied to Disc	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		SL4525 (rec$^+$)		SL4700 (rec$^-$)		TA1978 (uvrB$^+$)		TA1538 (uvrB$^-$)	
Negative Control									
Kanamycin	**100.0 µg**	**14**	**13**	**13**	**14**	**13**	**13**	**14**	**14**
Positive Controls									
MMS*	**10.0 mg**	**47**	**46**	**68**	**65**				
4-NQO†	**2.0 mg**					**12**	**12**	**24**	**23**
Folpet	0.1 mg	9	8	15	14	11	10	16	15
	0.5 mg	10	11	16	16	10	11	15	15

---

* MMS = Methylmethanesulfonate.

† 4-NQO = 4-Nitroquinoline-1-oxide.

Table 211

DIFFERENTIAL TOXICITY ASSAYS WITH SALMONELLA TYPHIMURIUM STRAINS

Experiment 2

Compound	Amount of Compound Applied to Disc	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		SL4525 (rec$^+$)		SL4700 (rec$^-$)		TA1978 (uvrB$^+$)		TA1538 (uvrB$^-$)	
Negative Control									
Kanamycin	100.0 µg	15	14	16	15	17	17	15	16
Positive Controls									
MMS*	10.0 mg	45	40	72	74				
4-NQO†	2.0 mg					12	12	28	26
Folpet	0.1 mg	10	10	14	14	9	10	14	14
	0.5 mg	10	10	15	15	10	10	15	15

---

* MMS = Methylmethanesulfonate.

† 4-NQO = 4-Nitroquinoline-1-oxide.

Table 212

MOUSE LYMPHOMA FORWARD MUTATION CONCENTRATION-RANGE ASSAY OF FOLPET IN THE ABSENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (µg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency ($\times 10^{-6}$)	Induced Mutation Frequency ($\times 10^{-6}$)
Solvent control (1% DMSO)	95.3	600	469	121	99.6	94.9	52	--
	104.7	600	473	103	100.4	105.1	44	--
Positive control (500 µg/ml EMS)	69.3	600	348	923	73.9	51.2	530	482
	71.9	600	332	972	70.5	50.7	586	538
Folpet								
0.01	115.5	600	469	145	99.6	115.0	62	14
0.02	104.3	600	401	126	85.1	88.8	63	15
0.03	104.0	600	464	143	98.5	102.5	62	14
0.05	104.7	600	464	155	98.5	103.1	67	19
0.06	104.7	600	408	138	86.6	90.7	68	20
0.07	97.7	600	385	145	81.7	79.9	75	27
0.08	93.1	600	504	161	107.0	99.6	64	16
0.09	93.7	600	444	133	94.3	88.3	60	12
0.1	93.2	600	561	142	119.1	111.0	51	3
0.2	57.5	600	702	176	149.0	85.7	50	2
0.3	30.8	600	505	237	107.2	33.0	94	46
0.4	10.9	600	483	312	102.5	11.2	129	81
0.5	4.3	600	364	338	77.3	3.3	186	138
0.6	3.4	NC*	--	--	--	--	--	--
0.7	2.6	NC	--	--	--	--	--	--
0.8	0.9	NC	--	--	--	--	--	--
0.9	0.9	NC	--	--	--	--	--	--
1	0.8	NC	--	--	--	--	--	--

---

*Not cloned.

Table 213

MOUSE LYMPHOMA FORWARD MUTATION CONCENTRATION-RANGE ASSAY OF FOLPET IN THE PRESENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency ($\times 10^{-6}$)	Induced Mutation Frequency ($\times 10^{-6}$)
Solvent control (1% DMSO)	106.4	600	546	192	108.9	115.8	70	--
	93.6	600	457	167	91.1	85.3	73	--
Positive control (5 μg/ml 3-MCA)	55.1	600	336	527	67.0	36.9	314	242
	63.3	600	336	622	67.0	42.4	370	298
Folpet								
1	102.8	600	429	160	85.5	87.9	75	3
2	102.8	600	582	164	116.1	119.3	56	--
5	99.5	600	507	166	101.1	100.6	65	--
10	79.3	600	489	193	97.5	77.3	79	7
20	21.7	600	494	287	98.5	21.2	116	44
40	0.3	NC*	--	--	--	--	--	--
80	0.5	NC	--	--	--	--	--	--
100	1.1	NC	--	--	--	--	--	--
150	2.4	NC	--	--	--	--	--	--
200	3.9	NC	--	--	--	--	--	--
300	PPT†	--	--	--	--	--	--	--
400	PPT	--	--	--	--	--	--	--
500	PPT	--	--	--	--	--	--	--
600	PPT	--	--	--	--	--	--	--
700	PPT	--	--	--	--	--	--	--
800	PPT	--	--	--	--	--	--	--
900	PPT	--	--	--	--	--	--	--
1000	PPT	--	--	--	--	--	--	--

---

*Not cloned.

†Persistent precipitate present after washdown procedure; samples discarded.

Table 214

MOUSE LYMPHOMA FORWARD MUTATION ASSAY OF FOLPET IN THE ABSENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (µg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency ($\times 10^{-6}$)	Induced Mutation Frequency ($\times 10^{-6}$)
Solvent control (1% DMSO)	100.0	600	541	108	95.8	95.8	40	--
	100.0	600	588	110	104.2	104.2	37	--
Positive control (500 µg/ml EMS)	66.4	600	372	981	65.9	43.8	527	488
	69.0	600	330	944	58.5	40.3	572	533
Folpet								
0.03	91.5	600	606	144	107.4	98.2	48	9
	91.5	600	611	190	108.2	99.0	62	23
0.06	72.5	600	623	89	110.4	80.0	29	--
	65.5	600	594	122	105.2	68.9	41	2
0.18	60.3	600	552	227	97.8	59.0	82	43
	60.3	600	532	184	94.2	56.8	69	30
0.27	42.1	600	551	261	97.6	41.1	95	56
	45.8	600	576	217	102.0	46.7	75	36
0.30	40.4	600	527	247	93.4	37.7	94	55
	38.9	600	520	217	92.1	35.8	83	44
0.36	31.5	600	501	289	88.8	28.0	115	76
	32.7	600	448	225	79.4	26.0	100	61
0.42	24.1	600	447	284	79.2	19.1	127	88
	25.2	600	449	319	79.5	20.0	142	103
0.45	22.8	600	410	265	72.6	16.6	129	90
	22.9	600	432	318	76.5	17.5	147	108
0.51	18.5	600	446	340	79.0	14.6	152	113
	16.3	600	381	306	67.5	11.0	161	122
0.60	9.9	600	334	320	59.2	5.9	192	153
	11.2	600	358	341	63.4	7.1	191	152

Table 215

MOUSE LYMPHOMA FORWARD MUTATION ASSAY OF FOLPET IN THE PRESENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (µg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency ($\times 10^{-6}$)	Induced Mutation Frequency ($\times 10^{-6}$)
Solvent control (1% DMSO)	97.6	600	537	321	102.9	100.4	120*	--
	102.4	600	507	384	97.1	99.5	151*	--
Positive control (6 µg/ml 3-MCA)	47.9	600	364	730	69.7	33.4	401	265
	56.8	600	383	645	73.4	41.7	337	201
Folpet								
0.5	104.1	600	608	429	116.5	121.3	141	5
	93.5	600	570	312	109.2	102.1	109	--
1	87.6	600	577	466	110.5	96.8	162	26
	92.9	600	566	425	108.4	100.7	150	14
5	90.1	600	568	413	108.8	98.0	145	9
	84.9	600	580	440	111.1	94.3	152	16
15	56.3	600	532	538	101.9	57.4	202	66
	56.0	600	480	554	92.0	51.5	231	95
18	50.9	600	557	691	106.7	54.3	248	112
	43.7	600	519	689	99.4	43.4	266	130
20	42.7	600	582	749	111.5	47.6	257	121
	40.2	600	475	749	91.0	36.6	315	179
22	--†	--	--	--	--	--	--	--
	31.0	600	433	765	83.0	25.7	353	217
24	21.5	600	300	598	57.5	12.4	399	263
	29.5	600	421	676	80.7	23.8	321	185
25	19.6	600	362	776	69.3	13.6	429	293
	16.5	600	301	620	57.7	9.5	412	276
30	4.4	600	153	568	29.3	1.3	742	606
	4.4	600	177	462	33.9	1.5	522	386

---

[*] The spontaneous mutation frequency of the solvent controls exceeds the assay standard of 100×10^{-6}. However, this assay has been included in the evaluation of folpet because: (1) the assay has a complete cytotoxic/survival range, over the concentrations tested, of nearly total kill to 100.0% survival; (2) the mutation frequency of the test samples ranges from several times the spontaneous mutation frequency to control level with relative total growth values of over 10.0%.

[†] Sample lost.

Table 216

UNSCHEDULED DNA SYNTHESIS OF FOLPET WITHOUT METABOLIC ACTIVATION

(dpm/μg DNA)

Retest

	Concentration of Compounds Tested						
	Folpet (M × 10^{-6})						4NQO (M)
Sample	0*	6.25	12.5	25	50	100	10^{-5}
1	53	31	29	12	1	1	1187
2	54	30	30	8	0	2	1334
3	55	74	34	7	2	0	1369
4	52	46	35	5	4	0	1351
5	39	39	35	2	8	3	1326
6	90	48	30	12	0	3	1425
Mean	57	45	32	8	2	2	1332
SD	17	16	3	4	3	1	79
SE	7	7	1	2	1	1	32

---

*Negative control and compound solvent, 0.5% DMSO.

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Table 217

UNSCHEDULED DNA SYNTHESIS ASSAY OF FOLPET WITH METABOLIC ACTIVATION
(dpm/μg DNA)
Retest

	Concentration of Compounds Tested						
	Folpet (M x 10^{-6})						DMN (M)
Sample	0*	3.7	11.1	33.3	100	300	5 x 10^{-2}
1	126	176	132	134	116	140	471
2	123	185	121	137	141	148	270
3	129	74	136	127	139	141	164
4	90	161	118	154	136	137	480
5	102	140	107	157	119	107	416
6	144	175	133	136	140	182	518
Mean	119	152	125	141	132	142	386
SD	20	41	11	12	11	24	139
SE	8	17	5	5	5	10	57

---

*Negative control and compound solvent, 0.5% DMSO.

Formetanate Hydrochloride

S. typhimurium Plate Incorporation, E. coli WP-2 (Tables 218, 219). Formetanate hydrochloride was tested twice on separate days over a concentration range of 500 to 10,000 µg/plate, using H_2O as the solvent. Toxicity was observed with all the S. typhimurium strains at concentrations greater than 7500 µg/plate. No reproducibly dose-related increase in the number of revertants was observed with formetanate hydrochloride in either test system.

S. cerevisiae D3 (Tables 220, 221). In this assay, formetanate hydrochloride was first tested over concentrations ranging from 0.05 to 0.5% and retested at concentrations ranging from 0.05 to 0.25%. No recombinogenic activity was obtained with formetanate hydrochloride. Toxicity was observed with concentrations greater than 0.25%.

E. coli/B. subtilis Relative Toxicity (Tables 222-224). Formetanate hydrochloride was first tested at concentrations of 1 and 5 mg/disc. Because poor zones of inhibition of growth were obtained at those concentrations, a second experiment was conducted at concentrations ranging from 5 to 20 mg/disc. A third experiment, with concentrations ranging from 10 to 50 mg/disc, was attempted to see if larger zones could be elicited. No reproducible, dose-related differential killing was observed with formetanate hydrochloride in these assays.

Table 218

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI WP2

FORMETANATE HYDROCHLORIDE

Experiment 1

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate										Tryptophan Revertants per Plate	
			TA1535		TA1537		TA1538		TA98		TA100		WP2	
Negative Control														
H₂O	-		9	10	2	6	7	8	31	38	112	159	20	21
	+		8	7	9	5	16	18	27	31	129	139	20	27
Positive Controls														
Sodium Azide	-	1.0	166	215							258	238		
9-Aminoacridine	-	50.0			359	341								
2-Nitrofluorene	-	5.0					549	555	342	345				
AF-2	-	0.1											420	420
2-Anthramine	-	1.0					15	10	19	31	109	114		
	+	1.0					321	333	342	296	643	725		
	-	2.5	12	15	8	5								
	+	2.5	186	163	154	139								
	-	10.0											18	17
	+	10.0											166	184
Formetanate HCl	-	500.0	14	7	9	5	19	17	26	33	159	129	30	27
	-	1,000.0	10	10	9	6	18	13	20	29	144	107	29	36
	-	2,500.0	10	6	7	5	16	19	20	28	140	114	32	36
	-	5,000.0	5	4	6	5	13	14	17	28	110	108	27	25
	-	7,500.0	5	5	8	3	18	15	20	20	158	116	33	28
	-	10,000.0	PP*	PP	PP	PP	PP	PP	16	18	72	123	24	29
	+	500.0	11	13	15	8	20	20	40	26	156	135	42	45
	+	1,000.0	8	7	14	14	21	19	39	20	143	123	54	48
	+	2,500.0	12	8	6	16	24	19	39	26	111	129	53	48
	+	5,000.0	6	9	6	12	13	17	20	16	93	98	32	31
	+	7,500.0	4	5	13	6	10	15	25	17	109	126	48	36
	+	10,000.0	4	4	10	12	9	12	15	14	129	130	37	33

---

*PP = Pinpoint colonies on background.

Table 219

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI WP2

FORMETANATE HYDROCHLORIDE

Experiment 2

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate TA1535		TA1537		TA1538		TA98		TA100		Tryptophan Revertants per Plate WP2	
Negative Control														
H_2O	-		13	13	7	5	7	7	19	15	161	152	23	24
	+		8	6	6	6	14	15	16	18	129	130	49	39
Positive Controls														
Sodium Azide	-	1.0	194	198							271	274		
9-Aminoacridine	-	50.0			509	450								
2-Nitrofluorene	-	5.0					661	589	298	300				
AF-2	-	0.1											776	625
2-Anthramine	-	1.0					7	7	25	12	161	128		
	+	1.0					260	356	268	269	439	444		
	-	2.5	7	15	17	8								
	+	2.5	273	224	184	178								
	-	10.0											25	24
	+	10.0											213	277
Formetanate HCl	-	500.0	17	17	6	4	12	8	21	18	178	160	25	18
	-	1,000.0	9	10	4	6	8	8	12	13	151	129	32	26
	-	2,500.0	14	17	3	6	6	4	8	14	161	155	21	18
	-	5,000.0	2	6	1	1	3	PP*	1	3	103	153	17	17
	-	7,500.0	PP	PP	PP	PP	PP	PP	PP	PP	27	31	31	16
	-	10,000.0	T†	T	T	T	T	T	T	T	PP	PP	10	13
	+	500.0	13	6	4	6	14	16	29	32	148	159	32	35
	+	1,000.0	8	10	4	8	15	15	28	21	137	154	24	32
	+	2,500.0	7	8	4	6	28	28	21	32	137	146	28	37
	+	5,000.0	7	7	5	7	30	32	13	18	125	130	34	40
	+	7,500.0	1	3	6	4	25	26	8	20	158	130	29	24
	+	10,000.0	PP	PP	3	2	6	11	4	7	73	86	33	30

---

*PP = Pinpoint colonies on background.

†T = toxic; cells all killed.

Table 220

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

FORMETANATE HYDROCHLORIDE

Experiment 1

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors: Cells per ml (x 10^{-7})	Survivors: Percent	Mitotic Recombinants: Per ml (x 10^{-3})	Mitotic Recombinants: Per 10^5 Survivors
Negative Control						
H_2O	-		8.0	100	2.0	2.5
	+		8.8	100	2.5	2.8
Positive Control						
1,2,3,4-Diepoxybutane	-	0.025	6.0	75	930	1600
	+	0.025	7.1	81	1300	1800
Formetanate HCl	-	0.05	7.5	94	5.0	6.7
	-	0.075	7.0	88	2.0	2.9
	-	0.1	6.0	75	6.0	10
	-	0.25	T*		T	T
	-	0.5	T		T	T
	+	0.05	7.0	88	3.0	4.3
	+	0.075	8.2	93	4.0	4.9
	+	0.1	7.3	83	2.0	2.7
	+	0.25	3.5	40	1.0	2.9
	+	0.5	T		T	T

---

*T = toxic.

Table 221

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

FORMETANATE HYDROCHLORIDE

Experiment 2

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml (x 10^{-7})	Percent	Per ml (x 10^{-3})	Per 10^5 Survivors
Negative Control						
H_2O	-		6.5	100	7.0	11
	+		6.6	100	5.0	7.6
Positive Control						
1,2,3,4-Diepoxybutane	-	0.025	4.4	76	990	2200
Formetanate HCl	-	0.05	6.9	110	7.0	10
	-	0.10	5.9	91	17	29
	-	0.15	2.9	45	9.0	31
	-	0.20	T*		T	T
	-	0.25	T		T	T
	+	0.05	6.3	95	7.0	11
	+	0.10	5.7	86	6.0	11
	+	0.15	6.6	100	6.0	9.1
	+	0.20	5.3	80	8.0	12
	+	0.25	2.9	44	6.0	21

---

*T = toxic.

Table 222

DIFFERENTIAL TOXICITY ASSAY

FORMETANATE HYDROCHLORIDE

Experiment 1

Compound	Amount of Compound Added per Plate	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		E. coli				B. subtilis			
		W3110 (polA$^+$)		p3478 (polA$^-$)		H17 (rec$^+$)		M45 (rec$^-$)	
Negative Controls									
Chloramphenicol	20 µg	20	21	23	22				
Kanamycin	100 µg					17	17	17	16
Positive Control									
Methylmethanesulfonate	10 mg	46	50	60	55	41	40	48	48
Formetanate HCl	1 mg	6	6	6	6	6	6	6	6
	5 mg	7	7	8	7	7	7	7	6

Table 223

DIFFERENTIAL TOXICITY ASSAY

FORMETANATE HYDROCHLORIDE

Experiment 2

Compound	Amount of Compound Added per Plate	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		E. coli				B. subtilis			
		W3110 ($polA^+$)		p3478 ($polA^-$)		H17 (rec^+)		M45 (rec^-)	
Negative Controls									
Chloramphenicol	20 μg	22	21	20	21				
Kanamycin	100 μg					17	18	19	18
Positive Control									
Methylmethanesulfonate	1 mg	25	26	33	34	18	21	25	28
Formetanate HCl	5 mg	6	7	7	7	7	7	6	6
	10	7	7	7	7	7	7	9	8
	20	9	8	8	9	8	7	9	9

Table 224

DIFFERENTIAL TOXICITY ASSAY
FORMETANATE HYDROCHLORIDE

Experiment 3

Compound	Amount of Compound Added per Plate	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		E. coli				B. subtilis			
		W3110 (polA$^+$)		p3478 (polA$^-$)		H17 (rec$^+$)		M45 (rec$^-$)	
Negative Controls									
Chloramphenicol	20 µg	22	22	22	21				
Kanamycin	100 µg					18	20	18	19
Positive Control									
Methylmethanesulfonate	1 mg	25	28	34	33	13	15	19	20
Formetanate HCl	10 mg	7	7	7	7	8	7	7	7
	25	7	7	8	9	10	9	9	8
	50	8	9	9	9	8	10	11	10

Malathion

S. typhimurium Plate Incorporation (Table 225). Malathion was previously tested in strains TA100, TA1535, TA1537 and TA1538 at concentrations up to 1000 µg/ml, and was negative both with and without activation (51). Under the present contract it was retested in strains TA98 and TA100 at concentrations up to 5000 µg/ml, and was again negative, both with and without activation.

E. coli WP-2. This compound was previously tested and found to be negative with and without activation (51).

S. cerevisiae D3 (Table 226). Malathion was previously tested at one concentration and found to be negative with and without activation (51). It was retested under the present contract over a range of concentrations, with similar results.

E. coli/B. subtilis Relative Toxicity. This compound was previously tested and found to be negative in both species (51).

UDS in WI-38 Cells. Malathion was previously tested and found negative with and without activation (51).

Mouse Dominant Lethal Test. This compound was previously tested and found negative (51).

Table 225

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

MALATHION

Retest 2

Compound	Metabolic Activation	Compound Added (µg/plate)	Histidine Revertants per Plate						
			TA1535	TA1537	TA1538	TA98		TA100	
Negative Control DMSO	-					24	16	135	110
	+					20	21	141	129
Positive Controls									
2-Anthramine	-	1.0				26		125	
	+	1.0				232		527	
Sodium Azide	-	0.5						286	
2-Nitrofluorene	-	5.0				394			
Malathion	-	500.0				8		124	
	-	1,000.0				18		121	
	-	2,000.0				16		110	
	-	3,000.0				9		122	
	-	4,000.0				16		104	
	-	5,000.0				19		110	
	+	500.0				20		136	
	+	1,000.0				17		124	
	+	2,000.0				25		123	
	+	3,000.0				16		134	
	+	4,000.0				20		130	
	+	5,000.0				19		122	

Table 226

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

MALATHION

Retest 1

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($x\ 10^{-7}$)	Percent	Per ml ($x\ 10^{-3}$)	Per 10^5 Survivors
Negative Control: DMSO	-		8.0	100	4.0	5.0
	+		7.9	100	3.5	4.4
Positive Control 1,2,3,4-Diepoxybutane	-	0.025	7.8	98	902.5	1157.1
	+	0.025	C*	C	C	C
Malathion	-	1	8.9	111	7.0	7.9
	-	2	7.8	98	7.0	9.0
	-	3	9.7	121	8.0	8.3
	-	4	C	C	C	C
	-	5	9.5	119	8.0	8.4
	+	1	7.5	95	7.0	9.3
	+	2	8.2	104	7.0	8.5
	+	3	C	C	C	C
	+	4	C	C	C	C
	+	5	8.5	108	6.0	7.1

* C, contaminated.

Methomyl

S. typhimurium Plate Incorporation, E. coli WP-2. Methomyl was previously tested and found to be negative in both of these systems with and without activation (51).

S. cerevisiae D3 (Table 227). This compound was previously tested at one concentration and found to be negative with and without activation (51). It was retested under the present contract over a range of concentrations, with similar results.

E. coli/B. subtilis, UDS in WI-38 Cells. Methomyl was previously tested in these systems and was found to be negative (51).

Table 227

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

METHOMYL

Retest

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml (x 10^{-7})	Percent	Per ml (x 10^{-3})	Per 10^5 Survivors
Negative Control: DMSO	-		7.7	100	3.5	4.6
	+		7.4	100	3.5	4.7
Positive Control	-	0.025	7.0	85	415	592.9
1,2,3,4-Diepoxybutane	+		7.3	82	412.5	565.1
Methomyl	-	1.0	7.9	103	6.0	7.5
	-	1.5	6.9	90	2.0	2.9
	-	2.0	8.0	104	6.0	7.5
	-	2.5	8.7	113	11.0	12.6
	-	3.0	9.2	119	10.0	10.9
	-	3.5	8.0	104	11.0	13.8
	+	1.0	7.8	105	5.0	6.4
	+	1.5	7.4	100	2.0	2.7
	+	2.0	9.3	126	2.0	2.2
	+	2.5	8.2	111	6.0	7.3
	+	3.0	7.5	101	10.0	13.3
	+	3.5	7.1	96	3.0	4.2

Monocrotophos

S. typhimurium Plate Incorporation (Tables 228-230). Monocrotophos was previously tested in strains TA100, TA1535, TA1537 and TA1538 at concentrations up to 1000 µg/plate and was found to be negative with and without activation (51). It was retested under the present contract in strains TA98, TA100, and TA1535 at concentrations up to 5000 µg/plate. It was found to be positive in strain TA100 only, both with and without activation.

E. coli WP-2. This compound was previously tested and found to be negative with and without activation (51).

S. cerevisiae D3 (Table 231). Monocrotophos was previously tested at one concentration and found to be positive with and without activation (51). Under the present contract it was retested over a range of concentrations, with similar findings.

S. cerevisiae D7 (Tables 232, 233). Monocrotophos was tested over concentrations ranging from 0.1 to 3.0% in the first experiment and 1.0 to 3.0% in the second. It induced mitotic crossing-over, gene conversion, and reverse mutation, with and without metabolic activation.

E. coli/B. subtilis Relative Toxicity. This compound was previously tested and found to be negative in both species (51). Reexamination of the results indicates that they are more appropriately interpreted as inconclusive.

S. typhimurium Relative Toxicity (Tables 234, 235). Monocrotophos was more toxic only to one of the repair-deficient strains (SL 4700).

SCE in CHO Cells (Tables 236-239). The monocrotophos solution was dissolved in DMSO at a 50% concentration. At a concentration of 0.5%, monocrotophos formed a cloudy precipitate in the culture medium. Table 236 presents the results of the cytotoxicity test of monocrotophos in CHO cell cultures without metabolic activation. At a concentration of 0.5%, the monocrotophos solution was cytotoxic to CHO cells; at a concentration of 0.1% the cultures yielded primarily M_1 metaphases were produced; and at concentrations of 0.1% and less, primarily M_2 metaphases were produced.

Table 238 presents the SCE frequencies in CHO cells exposed to monocrotophos without metabolic activation. One sample exposed to the 0.04% concentration of monocrotophos solution was lost because of a cracked flask. A one-way analysis of variance was conducted on SCE frequencies of the four lower concentrations of monocrotophos and of the negative controls. The variance between treatment groups was significantly greater than the variance within treatment groups. The response appeared to be concentration-related, with the maximum effect (approximately 1.5 to 1.6 times the negative control SCE frequencies) at 0.02%.

Table 239 presents the SCE frequencies in CHO cells exposed to monocrotophos with metabolic activation. The variance between treatment groups was significantly greater than the variance within treatment groups. The response appeared to be dose-related, with the maximum effect (approximately 1.9 to 2.2 times the negative control SCE frequencies) at 0.2%.

The test of monocrotophos with metabolic activation involved exposing CHO cells to a higher concentration range for a shorter time (0.0125 to 0.2% for 2 hours) than that used in the test without metabolic activation (0.0025 to 0.02% for 21.5 hours). Therefore, direct comparisons are inappropriate. However, we conclude that monocrotophos does not require metabolic activation to increase the SCE frequencies in the cells.

L5178Y Mouse Lymphoma (Tables 240-245). The initial concentration-range assays of monocrotophos in the absence and presence of the metabolic activation system were performed with 18 concentrations between 0.5 and 500 µg/ml (Tables 240 and 242). Little cytotoxicity was observed, both in the absence and in the presence of metablic activation. At the highest concentration of 500 µg/ml, the relative total growth values were 64.7% in the absence of the metabolic activation system and 82.5% in the presence of the metabolic activation system. Therefore, additional concentration-range assays were performed with 18 concentrations between 300 and 1000 µg/ml (Tables 241 and 243).

In the absence of the metabolic activation system, the combined results (Tables 240 and 241) showed that the concentration range to use in the mutagenesis assay was approximately 50 to 900 µg/ml (102.1% relative suspension growth at 50 µg/ml and 9.6% relative total growth at 900 µg/ml). In the presence of the metabolic activation system, the results in Table 243 showed that the approximate range to use in the mutagenesis assay was from less than 300 µg/ml to over 1000 µg/ml (64.5% relative total growth at 300 µg/ml and 44.0% relative total growth at 1000 µg/ml).

Table 244 presents the results of mutagenesis testing of monocrotophos in the absence of the S-9 metabolic activation system. The highest concentration of 900 µg/ml yielded an average relative total growth value of 22.0%. No significant depression in the relative total growth values was observed at 50 µg/ml, which yielded an average relative total growth value of 99.2%. From 50 to 900 µg/ml, a concentration-related increase in induced mutation frequency was observed. Average mutation frequencies of at least twice the spontaneous frequency were observed from 200 to 900 µg/ml (the eight highest concentrations).

Table 245 presents the results of mutagenesis testing of monocrotophos in the presence of the S-9 metabolic activation system. The highest concentration (1200 µg/ml) yielded an average relative total growth value of 39.3%. No significant depressions in the relative total growth values were observed at or below 240 µg/ml, which yielded an average relative total growth value of 9.26%. From 156 to 1200 µg/ml, a concentration-related increase in induced mutation frequency was observed. Average mutation frequencies of at least twice the spontaneous frequency were observed from 840 to 1200 µg/ml (the four highest concentrations).

These results indicate a mutagenic effect of monocrotophos at the thymidine kinase locus in L5178Y mouse lymphoma cells, both with and without the metabolic activation system. In both cases, monocrotophos increased the mutation frequency, in a concentration-related manner, above the spontaneous frequencies observed in the controls. The metabolic activation system was not required to elicit the mutagenic effect, but it did appear to decrease the cytotoxicity of monocrotophos.

UDS in WI38 Cells (Tables 246-248). Monocrotophos was previously tested and reported to be positive with and without activation (51). Because this compound was of particular interest to the EPA, it was retested under the present contract and found to be negative without activation, positive with activation. Considering the data as a whole, we conclude that the latter interpretation is the more appropriate.

Micronucleus Test (Tables 249, 250). The dosing and sacrifice schedules are shown in Table 249. The incidences of micronuclei in PCEs and the PCE to RBC ratios are given in Table 250. Monocrotophos was negative at all dose levels and sampling times ($\beta \leq 0.05$). This test of monocrotophos meets all the criteria for a decision that is nonmutagenic except that of maximum tolerated dose. Although the highest amount given was 2 X 90% of the reported LD_{50}, no fatalities occurred, and the mean weight loss for the dosed groups was less than that of the vehicle control group. There is, however, no trend apparent in the incidence of micronuclei among the treated groups. We conclude that monocrotophos is negative in this test system.

Mouse Dominant Lethal Test. This compound was previously tested in this system and was found to be negative (51).

Table 228

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

MONOCROTOPHOS

Retest 1

Compound	Metabolic Activation	Compound Added (µg/plate)	Histidine Revertants per Plate TA1535	TA1537	TA1538	TA98	TA100
Negative Control							
DMSO	-					10 13	100 105
	+					32 29	90 116
Positive Controls							
2-Anthramine	-	1.0				25	105
	+	1.0				273	488
Sodium Azide	-	0.5					474
2-Nitrofluorene	-	50.0				466	
Monocrotophos	-	500.0				16	91
	-	1,000.0				25	109
	-	2,000.0				20	128
	-	3,000.0				13	140
	-	4,000.0				15	154
	-	5,000.0				27	187
	+	500.0				20	97
	+	1,000.0				17	134
	+	2,000.0				20	161
	+	3,000.0				21	195
	+	4,000.0				20	234
	+	5,000.0				21	231

Table 229

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

MONOCROTOPHOS

Retest 2

Compound	Metabolic Activation	Compound Added (µg/plate)	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative Control							
DMSO	-		21				90
	+		9				84
Positive Controls							
2-Anthramine	-	1.0					167
	+	1.0					240
	-	2.5	17				
	+	2.5	108				
Sodium Azide	-	0.5	104				
	-	1.0					349
Monocrotophos	-	500.0	19				75
	-	1,000.0	12				99
	-	2,000.0	14				123
	-	3,000.0	9				129
	-	4,000.0	6				130
	-	5,000.0	5				126
	+	500.0	4				83
	+	1,000.0	12				109
	+	2,000.0	5				160
	+	3,000.0	8				175
	+	4,000.0	12				210
	+	5,000.0	11				264

Table 230

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

MONOCROTOPHOS

Retest 3

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate TA1535	TA1537	TA1538	TA98	TA100
Negative Control							
DMSO	-						148 142
	+						103 105
Positive Controls							
2-Anthramine	-	1.0					115
	+	1.0					456
Sodium Azide	-	1.0					647
Monocrotophos	-	1,000.0					155
	-	2,500.0					183
	-	5,000.0					220
	-	7,500.0					209
	-	10,000.0					280
	-	20,000.0					458
	+	1,000.0					130
	+	2,500.0					191
	+	5,000.0					245
	+	7,500.0					341
	+	10,000.0					332
	+	20,000.0					553

Table 231

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

MONOCROTOPHOS

Retest

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors: Cells per ml ($\times 10^{-7}$)	Survivors: Percent	Mitotic Recombinants: Per ml ($\times 10^{-3}$)	Mitotic Recombinants: Per 10^3 Survivors
Negative Control: DMSO	-		8.0	100	4.0	5.0
	+		7.9	100	3.5	4.4
Positive Control 1,2,3,4-Diepoxybutane	-	0.025	7.8	98	902.5	1157.1
	+	0.025	C*	C	C	C
Monocrotophos	-	1	8.8	110	13.0	14.8
	-	2	8.3	104	30.0	36.1
	-	3	10.2	128	49.0	48.0
	-	4	10.0	125	31.0	31.0
	-	5	6.9	86	64.0	92.8
	+	1	8.8	111	19.0	21.6
	+	2	7.9	100	35.0	44.3
	+	3	7.3	92	24.0	32.9
	+	4	7.8	99	45.0	58.0
	+	5	6.5	82	57.0	87.7

* C, contaminated.

Table 232

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D7

MONOCROTOPHOS

Experiment 1

			Survivors		Crossing-Over				Gene Conversion		Reverse Mutation	
					Mitotic Recombinants		Total Aberrants		Convertants		Revertants	
Compound	MA	% Conc.	Cells Per ml ($\times 10^{-7}$)	%	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors	Per ml ($\times 10^{-1}$)	Per 10^6 Survivors	Per ml ($\times 10^0$)	Per 10^7 Survivors
Negative Control												
DMSO	-	10	5.2	100	4.5	8.7	6.5	13	70	13	14	2.7
	-	10	5.6	100	1.5	2.7	11	20	66	12	16	2.9
Positive Control												
1,2,3,4-Diepoxybutane	-	0.013	3.9	75	96	250	250	640	1900	490	280	72
	+	0.013	3.7	66	92	250	370	1000	1600	430	330	89
Monocrotophos	-	0.1	4.2	81								
	-	0.5	4.2	81	0	0	12	29	53	13	12	2.9
	-	1.0	4.7	90	2.0	4.8	17	40	72	17	23	5.4
	-	2.0	4.0	77	1.0	2.1	29	62	65	14	31	6.6
	-	3.0	2.3	44	10	25	27	68	100	25	41	10
	+	0.1	5.3	95	19	83	57	250	210	91	64	28
	+	0.5	5.6	100	0	0	14	26	64	12	23	4.3
	+	1.0	5.1	91	0	0	15	27	55	9.8	22	3.9
	+	2.0	4.1	73	3.0	5.9	22	43	75	15	17	3.3
	+	3.0	3.8	68	8.0	2.0	21	51	80	20	34	8.3
					3.0	7.9	44	120	120	32	49	13

Table 233

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D7

MONOCROTOPHOS

Experiment 2

			Survivors		Crossing-Over				Gene Conversion		Reverse Mutation	
					Mitotic Recombinants		Total Aberrants		Convertants		Revertants	
Compound	MA	% Conc.	Cells Per ml (× 10^{-7})	%	Per ml (× 10^{-3})	Per 10^5 Survivors	Per ml (× 10^{-3})	Per 10^5 Survivors	Per ml (× 10^{-1})	Per 10^6 Survivors	Per ml (× 10^0)	Per 10^7 Survivors
Negative Control												
DMSO	-	10	3.5	100	1.0	2.9	8.0	2.3	61	17	20	5.7
	+	10	3.5	100	1.0	2.9	11	31	54	15	22	6.3
Positive Control												
1,2,3,4-Diepoxybutane	-	0.013	1.5	43	67	450	340	2300	1000	670	450	300
	+	0.013	2.3	66	44	190	370	1600	1200	520	410	180
Monocrotophos	-	1.0	2.7	74	7.0	26	34	130	210	78	69	26
	-	1.5	2.3	66	7.0	30	49	210	240	100	69	30
	-	2.0	1.7	49	13	76	72	420	260	150	70	41
	-	2.5	0.77	22	18	230	39	510	150	190	42	55
	-	3.0	0.43	12	12	280	46	1100	56	130	26	60
	+	1.0	3.0	86	5.0	17	26	87	130	43	58	19
	+	1.5	3.3	94	0	0	19	58	210	64	88	27
	+	2.0	3.4	97	2.0	5.9	15	44	240	71	74	22
	+	2.5	2.1	60	9.0	43	38	180	320	150	65	31
	+	3.0	2.0	57	9.0	45	91	460	210	110	79	40

Table 234

DIFFERENTIAL TOXICITY ASSAYS WITH SALMONELLA TYPHIMURIUM STRAINS

Experiment 1

		Zone of Inhibition of Growth (in mm, disc = 6 mm)							
Compound	Amount of Compound Applied to Disc	SL4525 (rec$^+$)		SL4700 (rec$^-$)		TA1978 (uvrB$^+$)		TA1538 (uvrB$^-$)	
Negative Control									
Kanamycin	100.0 µg	14	13	13	14	13	13	14	14
Positive Controls									
MMS*	10.0 mg	47	46	68	65				
4-NQO†	2.0 mg					12	12	24	23
Monocrotophos	5.0 µl	9	8	15	14	9	9	9	9
	10.0 µl	10	9	16	17	10	10	11	11

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* MMS = Methylmethanesulfonate.

† 4-NQO = 4-Nitroquinoline-1-oxide.

Table 235

DIFFERENTIAL TOXICITY ASSAYS WITH SALMONELLA TYPHIMURIUM STRAINS

Experiment 2

Compound	Amount of Compound Applied to Disc	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		SL4525 (rec⁺)		SL4700 (rec⁻)		TA1978 (uvrB⁺)		TA1538 (uvrB⁻)	
Negative Control									
Kanamycin	**100.0 µg**	**15**	**14**	**16**	**15**	**17**	**17**	**15**	**16**
Positive Controls									
MMS*	**10.0 mg**	**45**	**40**	**72**	**74**				
4-NQO†	**2.0 mg**					**12**	**12**	**28**	**26**
Monocrotophos	5.0 µl	9	9	11	12	8	9	7	9
	10.0 µl	10	9	15	16	10	9	11	11

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* MMS = Methylmethanesulfonate.

† 4-NQO = 4-Nitroquinoline-1-oxide.

Table 236

CYTOTOXICITY TEST OF CHINESE HAMSTER OVARY CELLS EXPOSED TO MONOCROTOPHOS FOR 21.5 HOURS WITH METABOLIC ACTIVATION

Treatment	Number of Divisions in Bromodeoxyuridine (BrdU)[*]
Negative control (1% DMSO)	M_2
Monocrotophos solution (%)	
0.0008	M_2
0.004	M_2
0.02	$M_2 > M_1$[†]
0.1	M_1
0.5[‡]	--[§]
Positive control (10^{-3} M ethyl methanesulfonate)	$M_2 > M_1$[†]

---

[*] M_1, one division in BrdU; M_2, two divisions in BrdU.

[†] The cell type to the left of the > sign was in the majority.

[‡] Chemical formed cloudy precipitate in the culture medium.

[§] This culture was not harvested because of excessive cytotoxicity.

Table 237

CYTOTOXICITY TEST OF CHINESE HAMSTER OVARY CELLS EXPOSED TO MONOCROTOPHOS FOR 2 HOURS WITH METABOLIC ACTIVATION

Treatment	Number of Divisions in Bromodeoxyuridine (BrdU)[*]
Negative control (1% DMSO)	M_2
Monocrotophos solution (%)	
0.0008	$M_2 > M_1$[†]
0.004	$M_2 >> M_1$[‡]
0.02	$M_2 >> M_1$[‡]
0.1	M_2
0.5	M_1
Positive control (10^{-3} M dimethylnitrosamine)	M_2

---

[*] M_1, one division in BrdU; M_2, two divisions in BrdU.

[†] The cell type to the left of the > sign was in the majority.

[‡] The cell type to the left of the >> was in great excess.

Table 238

SISTER CHROMATID EXCHANGE (SCE) FREQUENCIES IN CHINESE HAMSTER OVARY (CHO) CELLS EXPOSED TO MONOCROTOPHOS FOR 21.5 HOURS WITHOUT METABOLIC ACTIVATION

Treatment	Cyto-geneticist*	No. of SCEs	No. of Chromosomes	Chromosomes/Cell (Mean ± SD)	SCEs/Cell† (Mean ± SE)	SCEs/Chromosome† (Mean ± SE)
Negative control (1% DMSO)	A	321	478	19.12 ± 0.60	12.84 ± 0.72	0.672 ± 0.037
	B	294	481	19.24 ± 0.88	11.76 ± 0.69	0.611 ± 0.036
Monocrotophos solution (%)						
0.0025	A	285	472	18.80 ± 0.83	11.40 ± 0.68	0.604 ± 0.036
	B	283	485	19.40 ± 0.65	11.32 ± 0.67	0.584 ± 0.035
0.005	A	326	478	19.12 ± 0.53	13.04 ± 0.72	0.682 ± 0.038
	B	350	494	19.76 ± 0.66	14.00 ± 0.75	0.709 ± 0.038
0.01	A	404	483	19.32 ± 0.56	16.16 ± 0.80	0.836 ± 0.042
	B	373	498	19.92 ± 0.57	14.92 ± 0.77	0.749 ± 0.039
0.02	A	476	480	19.20 ± 1.00	19.04 ± 0.87	0.992 ± 0.045
	B	476	489	19.56 ± 1.04	19.04 ± 0.87	0.973 ± 0.045
0.04		--‡				
Positive control (10^{-3} M ethyl methanesulfonate)	A	788	476	19.04 ± 0.74	31.52 ± 1.12	1.655 ± 0.059
	B	669	486	19.44 ± 0.65	26.76 ± 1.03	1.376 ± 0.053

---

*Each cytogeneticist analyzed 25 cells per sample.

†A one-way analysis of variance comparing the SCE frequencies in CHO cells exposed to monocrotophos and to the negative control indicated that the variance between treatment groups was significantly greater ($P < 0.0005$ for SCEs/cells; $P < 0.001$ for SCEs/chromosome) than the variance within treatment groups.

‡One sample was lost because of a cracked flask.

Table 239

SISTER CHROMATID EXCHANGE (SCE) FREQUENCIES IN CHINESE HAMSTER OVARY (CHO) CELLS EXPOSED TO MONOCROTOPHOS FOR 2

Treatment	Cyto-geneticist*	No. of SCEs	No. of Chromosomes	Chromosomes/Cell (Mean ± SD)	SCEs/Cell† (Mean ± SE)	SCEs/ Chromosome† (Mean ± SE)
Negative control (1% DMSO)						
	A	354	482	19.28 ± 0.74	14.16 ± 0.75	0.734 ± 0.039
	B	306	492	19.68 ± 0.95	12.24 ± 0.70	0.622 ± 0.036
Monocrotophos solution (%)						
0.0125	A	369	485	19.40 ± 0.87	14.76 ± 0.77	0.761 ± 0.040
	B	354	498	19.92 ± 1.08	14.16 ± 0.75	0.711 ± 0.038
0.025	A	388	487	19.48 ± 0.96	15.52 ± 0.79	0.797 ± 0.040
	B	396	504	20.16 ± 1.07	15.84 ± 0.80	0.786 ± 0.040
0.05	A	439	494	19.76 ± 0.60	17.56 ± 0.84	0.889 ± 0.042
	B	423	491	19.64 ± 0.81	16.92 ± 0.82	0.862 ± 0.042
0.1	A	636	488	19.52 ± 0.87	25.44 ± 1.01	1.303 ± 0.052
	B	493	502	20.08 ± 0.95	19.72 ± 0.89	0.982 ± 0.044
0.2	A	675	488	19.52 ± 1.00	27.00 ± 1.04	1.383 ± 0.053
	B	680	496	19.84 ± 1.14	27.20 ± 1.04	1.371 ± 0.053
Positive control (10^{-3} M dimethylnitrosamine)						
	A	1059	495	19.80 ± 1.00	42.36 ± 1.30	2.139 ± 0.066
	B	979	507	20.28 ± 0.84	39.16 ± 1.25	1.931 ± 0.062

---

[*] Each cytogeneticist analyzed 25 cells per sample.

[†] A one-way analysis of variance comparing the SCE frequencies in CHO cells exposed to monocrotophos and to the negative control indicated that the variance between treatment groups was significantly greater ($P < 0.005$) than the variance within treatment groups.

Table 240

MOUSE LYMPHOMA FORWARD MUTATION CONCENTRATION-RANGE ASSAY OF MONOCROTOPHOS IN THE ABSENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency (× 10⁻⁶)	Induced Mutation Frequency (× 10⁻⁶)
Solvent control (1% DMSO)	97.4	600	572	130	93.4	91.0	45	--
	102.6	600	653	129	106.6	109.4	40	--
Positive control (500 μg/ml EMS)	68.1	60	343	1058	56.0	38.1	617	574
	71.0	60	278	1110	45.4	32.2	799	756
Monocrotophos								
0.5	107.6	NC*	--	--	--	--	--	--
1	107.6	NC	--	--	--	--	--	--
2	102.1	NC	--	--	--	--	--	--
5	107.5	NC	--	--	--	--	--	--
8	107.6	NC	--	--	--	--	--	--
10	102.1	NC	--	--	--	--	--	--
20	102.1	NC	--	--	--	--	--	--
30	102.1	NC	--	--	--	--	--	--
50	102.1	NC	--	--	--	--	--	--
60	97.4	NC	--	--	--	--	--	--
80	107.6	NC	--	--	--	--	--	--
100	107.6	NC	--	--	--	--	--	--
150	102.7	NC	--	--	--	--	--	--
200	92.8	600	712	181	116.2	107.9	51	8
250	88.7	600	632	226	103.2	91.5	72	30
300	85.2	600	634	--†	103.5	88.2	--	--
400	75.0	600	525	--†	85.7	64.3	--	--
500	68.9	600	575	319	93.9	64.7	111	68

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*Not cloned.

†Sample lost.

Table 241

REPEAT MOUSE LYMPHOMA FORWARD MUTATION CONCENTRATION-RANGE ASSAY OF MONOCROTOPHOS IN THE ABSENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency (× 10^{-6})	Induced Mutation Frequency (× 10^{-6})
Solvent control (1% DMSO)	97.4	600	622	193	113.8	110.9	62	--
	102.6	600	471	122	86.2	88.4	52	--
Positive control (500 μg/ml EMS)	30.5	600	112	674	20.5	6.3*	1204	1147
	30.5	600	95	714	17.4	5.3*	1503	1446
Monocrotophos								
300	62.3	600	597	229	109.2	68.1	77	20
400	57.7	600	704	265	128.8	74.3	75	18
500	51.8	600	522	274	95.5	49.5	105	48
600	41.0	600	420	324	76.9	31.5	154	97
650	40.3	600	442	401	80.9	32.6	181	124
700	33.5	600	560	450	102.5	34.3	161	104
730	31.0	600	473	421	86.6	26.8	178	121
750	29.9	600	533	457	97.5	29.2	171	114
780	27.3	600	356	448	65.1	17.8	252	195
800	25.7	600	404	430	73.9	19.0	213	156
830	24.1	600	350	499	64.0	15.4	285	228
850	23.9	600	324	410	59.3	14.2	253	196
880	21.6	600	272	351	49.8	10.8	258	201
900	21.1	600	248	428	45.4	9.6	345	288
930	19.9	600	261	391	47.8	9.5	300	243
950	21.2	600	269	371	49.2	10.4	276	219
980	15.9	600	216	457	39.5	6.3	423	366
1000	18.5	600	203	442	37.1	6.9	435	378

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*Apparent chemical dilution error of EMS positive control; results included because valid assay criteria met.

Table 242

MOUSE LYMPHOMA FORWARD MUTATION CONCENTRATION-RANGE ASSAY OF MONOCROTOPHOS IN THE PRESENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency ($\times 10^{-6}$)	Induced Mutation Frequency ($\times 10^{-6}$)
Solvent control (1% DMSO)	96.2	600	581	223	106.2	102.2	77	--
	103.8	600	513	224	93.8	97.3	87	--
Positive control (5 μg/ml 3-MCA)	54.9	600	692	591	126.5	69.5	171	89
	59.2	600	--*	582	--	--	--	--
Monocrotophos								
0.5	105.4	NC†	--	--	--	--	--	--
1	117.8	NC	--	--	--	--	--	--
2	88.5	NC	--	--	--	--	--	--
5	95.4	NC	--	--	--	--	--	--
8	100.0	NC	--	--	--	--	--	--
10	99.0	NC	--	--	--	--	--	--
20	111.2	NC	--	--	--	--	--	--
30	100.0	NC	--	--	--	--	--	--
50	91.8	NC	--	--	--	--	--	--
60	95.4	NC	--	--	--	--	--	--
80	91.0	NC	--	--	--	--	--	--
100	83.4	NC	--	--	--	--	--	--
150	68.8	NC	--	--	--	--	--	--
200	74.2	600	696	293	127.2	94.4	84	2
250	96.2	600	485	272	88.7	85.3	112	30
300	84.4	600	698	307	127.6	107.7	88	6
400	86.5	600	636	350	116.3	100.6	110	28
500	85.5	600	528	368	96.5	82.5	139	57

---

*Sample lost.

†Not cloned.

Table 243

REPEAT MOUSE LYMPHOMA FORWARD MUTATION CONCENTRATION-RANGE ASSAY OF MONOCROTOPHOS IN THE PRESENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency ($\times 10^{-6}$)	Induced Mutation Frequency ($\times 10^{-6}$)
Solvent control (1% DMSO)	98.3	600	538	308	101.3	99.6	114	--
	101.7	600	524	273	98.7	100.4	104	--
Positive control (5 μg/ml 3-MCA)	39.7	600	232	463	43.7	17.3	399	290
	36.5	600	219	451	41.2	15.1	412	303
Monocrotophos								
300	86.9	600	394	360	74.2	64.5	183	74
400	86.4	600	386	443	72.7	62.8	230	121
500	82.4	600	441	433	83.1	68.4	196	87
600	81.1	600	332	430	62.5	50.7	259	150
650	86.4	600	471	506	88.7	76.6	215	106
700	78.4	600	400	417	75.3	59.1	209	100
730	80.0	600	368	378	69.3	55.4	205	96
750	75.5	600	303	412	57.1	43.1	272	163
780	84.3	600	360	442	67.8	57.2	246	137
800	83.9	600	310	443	58.4	49.0	286	177
830	80.4	600	300	470	56.5	45.4	313	204
850	86.4	600	487	448	91.7	79.2	184	75
880	93.3	600	289	381	54.4	50.8	264	155
900	73.0	600	339	448	63.8	46.6	264	155
930	82.2	600	289	497	54.4	44.7	344	235
950	67.9	600	315	441	59.3	40.3	280	171
980	77.9	600	268	405	50.5	39.3	302	193
1000	66.6	600	351	483	66.1	44.0	275	166

Table 244

MOUSE LYMPHOMA FORWARD MUTATION ASSAY OF MONOCROTOPHOS IN THE ABSENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency ($\times 10^{-6}$)	Induced Mutation Frequency ($\times 10^{-6}$)
Solvent control (1% DMSO)	96.9	600	554	79	99.2	96.1	29	--
	103.1	600	563	76	100.8	103.9	27	--
Positive control (500 μg/ml EMS)	66.2	600	312	973	55.9	37.0	624	596
	89.1	600	313	978	56.0	49.9	625	597
Monocrotophos								
50	95.6	600	614	140	109.9	105.1	46	18
	100.3	600	519	77	92.9	93.2	30	2
100	86.0	600	544	148	97.4	83.8	54	26
	90.2	600	582	120	104.2	94.0	41	13
200	78.2	600	579	154	103.7	81.1	53	25
	78.5	600	535	170	95.8	75.2	64	36
300	68.5	600	489	161	87.6	60.0	66	38
	82.1	600	535	131	95.8	78.6	49	21
400	75.6	600	562	160	100.6	76.1	57	29
	69.5	600	570	198	102.1	70.9	69	41
500	52.0	600	499	210	89.3	46.5	84	56
	52.0	600	515	172	92.2	47.9	67	39
600	50.1	600	552	247	98.8	49.5	89	61
	46.6	600	541	266	96.9	45.1	98	70
700	50.1	600	478	214	85.6	42.9	90	62
	37.2	600	464	220	83.1	30.9	95	67
750	39.1	600	438	236	78.4	30.7	108	80
	35.3	600	490	254	87.7	31.0	104	79
900	31.8	600	447	293	80.0	25.5	131	103
	26.1	600	394	298	70.5	18.4	151	123

Table 245

MOUSE LYMPHOMA FORWARD MUTATION ASSAY OF MONOCROTOPHOS IN THE PRESENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency ($\times 10^{-6}$)	Induced Mutation Frequency ($\times 10^{-6}$)
Solvent control (1% DMSO)	92.4	600	459	169	97.9	90.4	74	--
	107.6	600	479	158	102.1	109.9	66	--
Positive control (5 μg/ml 3-MCA)	49.5	600	296	683	63.1	31.2	461	391
	46.8	600	343	639	73.1	34.2	373	303
Monocrotophos								
156	80.9	600	516	202	110.0	89.0	78	8
	85.6	600	527	194	112.4	96.2	74	4
240	84.2	600	512	216	109.2	91.9	84	14
	81.6	600	536	214	114.3	93.3	80	10
360	79.1	600	--*	251	--	--	--	--
	81.6	600	487	194	103.8	84.7	80	10
480	82.8	600	491	260	104.7	86.7	106	36
	79.1	600	437	226	93.2	73.7	103	33
600	88.3	600	494	256	105.3	93.0	104	34
	77.2	600	447	262	95.3	73.6	117	47
720	70.4	600	457	328	97.4	68.6	144	74
	71.3	600	458	304	97.7	69.6	133	63
840	68.0	600	437	342	93.2	63.4	157	87
	76.8	600	442	325	94.2	72.4	147	77
1008	60.3	600	456	323	97.2	58.6	142	72
	63.0	600	469	374	100.0	63.0	159	89
1080	63.3	600	377	--*	80.4	50.9	--	--
	76.8	600	420	390	89.6	68.8	186	116
1200	54.2	600	353	427	75.3	40.8	242	172
	51.7	600	343	377	73.1	37.8	220	150

---

*Sample lost.

Table 246

UNSCHEDULED DNA SYNTHESIS ASSAY OF MONOCROTOPHOS WITHOUT METABOLIC ACTIVATION

(dpm/μg DNA)

Retest 1

Sample	Concentration of Compounds Tested						
	Monocrotophos (M x 10^{-4})						4NQO (M) 10^{-5}
	0*	1.2	3.7	11.1	33.3	100	
1	101	109	213	69	205	43	1341
2	99	78	82	140	133	70	1297
3	122	148	216	158	116	100	1243
4	153	71	84	87	118	68	1553
5	91	95	127	75	156	73	1239
6	--†	150	121	111	176	68	1161
Mean	113	109	141	107	150	71	1305
SD	25	34	60	36	35	18	135
SE	11	14	25	15	14	7	55

---

*Negative control and compound solvent, 0.5% DMSO.

†Sample lost.

Table 247

UNSCHEDULED DNA SYNTHESIS ASSAY OF MONOCROTOPHOS WITHOUT METABOLIC ACTIVATION

(dpm/μg DNA)

Retest 2

Sample	Concentration of Compounds Tested						
	Monocrotophos (M x 10^{-4})						4NQO (M) (10^{-5})
	0*	1.2	3.7	11	33	100	
1	96	66	67	114	75	81	1890
2	98	113	58	98	90	71	1954
3	74	63	62	74	95	77	2031
4	89	64	72	80	142	56	2077
5	109	71	83	143	49	53	2219
6	127	168	56	104	73	67	1281
Mean	99	91	67	102	87	67	1909
SD	18	42	10	25	31	11	327
SE	7	17	4	10	13	4	134

---

*Negative control and compound solvent, 0.5% DMSO.

Table 248

UNSCHEDULED DNA SYNTHESIS ASSAY OF MONOCROTOPHOS WITH METABOLIC ACTIVATION
(dpm/μg DNA)
Retest

	Concentration of Compounds Tested						
	Monocrotophos ($M \times 10^{-4}$)						DMN (M)
Sample	0*	1.2	3.7	11.1	33.3	100	5×10^{-2}
1	120	67	88	77	120	164	452
2	116	100	105	102	150	114	444
3	81	120	98	79	150	125	516
4	117	67	100	78	110	155	466
5	63	91	103	95	117	157	382
6	120	64	81	74	102	170	359
Mean	103	85	96	84	125	148	436
SD	25	22	9	11	20	23	58
SE	10	9	4	5	8	9	24

---

*Negative control and compound solvent, 0.5% DMSO.

Table 249

DOSAGE AND SACRIFICE SCHEDULE

Compound	No. Animals	Dose		Mean Body Weight Change (g)	No. Sacrificed			No. Fatalities
		0 Hr	24 Hr		48 Hr	72 Hr	96 Hr	
TMP--Positive control	8	1 g/kg	1 g/kg	- 1.6	8	--	--	0
DMSO--Vehicle control	24	5 ml/kg	5 ml/kg	- 1.9	8	8	8	0
Monocrotophos Low Dose	24	2 mg/kg	2 mg/kg	- 0.5	8	8	8	0
Medium Dose	24	4 mg/kg	4 mg/kg	- 1.3	8	8	8	0
High Dose	24	8 mg/kg	8 mg/kg	- 1.2	8	8	8	0

Table 250

INCIDENCE OF MICRONUCLEATED POLYCHROMATOPHILIC ERYTHROCYTES*
(500 PCEs Scored per Animal)

	48 Hour			72 Hour			96 Hour		
Dose	Animal No.	PCE with MN	PCE/RBC	Animal No.	PCE with MN	PCE/RBC	Animal No.	PCE with MN	PCE/RBC
DMSO 2 x 5 ml/kg Vehicle control	78ø	2	1.55	77ø	2	0.48	79ø	2	1.72
	78r	1	1.41	77r	0	0.55	79r	1	1.08
	78ℓ	2	2.58	77ℓ	0	0.73	79ℓ	2	1.01
	78b	2	2.13	77b	3	0.42	79b	0	1.27
	81ø	0	1.83	80ø	1	1.18	82ø	0	0.82
	81r	1	1.96	80r	0	1.61	82r	1	1.20
	81ℓ	1	1.33	80ℓ	0	1.83	82ℓ	1	0.70
	81b	0	1.25	80b	1	1.40	82b	1	0.98
	Total	9			7			8	
	Average		1.76			1.03			1.10
Monocrotophos 2 x 2 mg/kg	83ø	1	1.10	84ø	0	1.85	85ø	1	1.32
	83r	1	1.62	84r	1	0.84	85r	0	1.43
	83ℓ	0	1.16	84ℓ	0	1.09	85ℓ	0	2.14
	83b	2	1.53	84b	0	1.06	85b	1	1.10
	86ø	1	3.13	87ø	2	1.49	88ø	2	0.89
	86r	1	1.94	87r	1	2.79	88r	2	1.37
	86ℓ	1	1.23	87ℓ	2	1.48	88ℓ	1	0.79
	86b	1	1.12	87b	0	0.94	88b	1	1.12
	Total	8			6			8	
	Average		1.60			1.44			1.27
Monocrotophos 2 x 4 mg/kg	92ø	0	1.92	89ø	0	0.98	90ø	0	1.55
	92r	0	1.24	89r	1	2.08	90r	1	2.22
	92ℓ	2	1.30	89ℓ	1	1.65	90ℓ	0	1.33
	92b	1	0.82	89b	0	1.38	90b	2	0.92
	94ø	2	1.50	91ø	0	1.92	93ø	0	1.37
	94r	0	1.28	91r	1	1.64	93r	2	0.95
	94ℓ	0	0.63	91ℓ	1	0.88	93ℓ	1	1.21
	94b	0	2.02	91b	0	1.15	93b	1	1.14
	Total	5			4			7	
	Average		1.34			1.46			1.34

continued

Table 250 (Concluded)

Dose	48 Hour			72 Hour			96 Hour		
	Animal No.	PCE with MN	PCE/RBC	Animal No.	PCE with MN	PCE/RBC	Animal No.	PCE with MN	PCE/RBC
Monocrotophos 2 x 8 mg/kg	97ø	1	1.05	96ø	0	0.89	95ø	0	2.61
	97r	1	0.94	96r	1	1.18	95r	0	0.78
	97ℓ	1	1.00	96ℓ	0	0.84	95ℓ	1	0.78
	97b	2	0.71	96b	0	0.49	95b	1	0.78
	98ø	0	1.14	99ø	0	1.27	100ø	1	0.84
	98r	2	2.47	99r	1	1.04	100r	1	0.57
	98ℓ	0	0.74	99ℓ	1	0.87	100ℓ	0	0.40
	98b	2	0.56	99b	0	1.23	100b	1	0.60
	Total	9			3			5	
	Average		1.08			0.98			0.92
TMP† 2 x 1000 mg/kg	50ø	4	1.91						
	50r	12	0.88						
	50ℓ	7	1.42						
	50b	8	1.14						
	51ø	7	1.44						
	51r	4	1.16						
	51ℓ	4	1.06						
	51b	4	0.82						
	Total	50							
	Average		1.23						

---

*PCE = polychromatophilic erythrocyte; MN = micronucleus; RBC = mature erythrocyte.

†This positive control group is the same group reported with the micronucleus test of acephate, which was done at the same time as this test.

Monuron

S. typhimurium Plate Incorporation, E. coli WP-2, S. cerevisiae D3. Monuron was previously tested and found to be negative, with and without activation in these three systems (51).

S. cerevisiae D7 (Tables 251, 252). No reproducible increase in the number of aberrant-type colonies, tryptophan revertants, or isoleucine revertants was observed when monuron was tested up to a concentration of 4.0%.

E. coli/B. subtilis Relative Toxicity. Monuron was previously tested and found to be negative in both species (51).

SCE in CHO Cells (Tables 253-256). Monuron was dissolved in DMSO at a concentration of 325 mg/ml. Table 253 presents the results of the cytotoxicity test of monuron in CHO cell cultures without metabolic activation. At concentrations of 26 μg/ml and above, the cultures yielded primarily M_1 metaphases. At 5.2 μg/ml, M_2 metaphases were in excess. Table 254 presents the results of the cytotoxicity test of monuron in CHO cell cultures with metabolic activation. At a concentration of 2522 μg/ml, primarily M_1 metaphases were produced; at concentrations of 504 μg/ml and less, primarily M_2 metaphases were produced.

Table 255 presents the SCE frequencies in CHO cells exposed to monuron without metabolic activation. A one-way analysis of variance was conducted on SCE frequencies of the five concentrations of monuron and of the negative controls. The variance between treatment groups was not significantly greater than the variance within treatment groups. Table 256 presents the SCE frequencies in CHO cells exposed to monuron with metabolic activation. The variance between treatment groups was significantly greater than the variance within treatment groups. The response appeared to be dose-related. The maximum effect (approximately 2.1 times the negative control SCE frequencies) was seen at 2000 μg/ml.

The test of monuron with metabolic activation involved exposing CHO cells to a higher concentration range for a shorter time (125 to 2000 μg/ml for 2 hours) than that used in the test without metabolic activation (0.625 to 10 μg/ml for 21.5 hours). Therefore, direct comparisons are inappropriate. However, we conclude that monuron requires metabolic activation to increase the SCE frequencies in CHO cells.

L5178Y Mouse Lymphoma (Tables 257-260). The results of the concentration-range testing of monuron in the absence of the metabolic activation system (Table 257) indicated that the range to use in the mutagenesis assay was approximately 80 to somewhat greater than 800 μg/ml (98.1% relative total growth at 80 μg/ml and 23.9% relative total growth at 800 μg/ml). Concentration-range test results of monuron in the presence of the metabolic activation system (Table 258) showed that the approximate range to use in the mutagenesis assay was 2 to 40 μg/ml (98.5% relative total growth at 2 μg/ml and 11.8% relative total growth at 40 μg/ml).

Table 259 presents the results of mutagenesis testing of monuron in the absence of the metabolic activation system. The highest concentration tested (1100 μg/ml), yielded an average relative total growth value of 28.5%. No significant depression in relative total growth was observed at a concentration of 100 μg/ml or less. At 100 μg/ml the average relative total growth was 95.4%. Over the tested range, there was some evidence of a concentration-related increase in induced mutation frequency. Average mutation frequencies of at least twice the spontaneous frequency were observed at 748, 1001, 1067, and 1100 μg/ml.

Table 260 presents the results of mutagenesis testing of monuron in the presence of the metabolic activation system. The highest concentration tested (60 μg/ml), yielded an average relative total growth of 21.4%. The lowest concentration tested (6 μg/ml), yielded an average relative total growth of 85.1%. Over the tested range, a concentration-related increase in induced mutation frequency was observed. Average frequencies of at least twice the spontaneous frequency were observed from 21 to 60 μg/ml (eight concentrations).

These combined results indicate a mutagenic effect of monuron at the thymidine kinase locus in L5178Y mouse lymphoma cells, both with and without the metabolic activation system. In both cases, monuron increased the mutation frequency, in a concentration-related manner, above the frequencies observed with the solvent controls. The presence of the metabolic activation system apeared to enhance the cytotoxic and mutagenic effect of monuron.

UDS in WI-38 Cells (Tables 261-263). Monuron was previously tested and reported to be negative without activation, positive with activation (51). Because the control values in that test appeared somewhat low, it was retested under the present contract. The results indicate that monuron is negative with and without activation. Considering the results as a whole, we conclude that the latter interpretation is appropriate.

Micronucleus Test (Tables 264, 265). The dosing and sacrifice schedules are shown in Table 264. The incidences of micronuclei in PCEs and the PCE to RBC ratios obtained in this study are given in Table 265.

Monuron was positive in the micronucleus test at 48 hours in the low- and medium-dose groups and at 96 hours in the low-dose group ($\alpha < 0.05$). The medium-dose group was negative at 96 hours ($\beta < 0.05$). The remaining treatment groups all fell in the "no-decision" range (α and $\beta > 0.05$). The vehicle control groups were negative at all times ($\beta < 0.05$). The PCE-to-RBC ratios were within normal limits. Because three dose-time groups were in the positive range, monuron meets the criteria for mutagenesis in the mouse micronucleus test.

The discrepancy in results between this test and a previously reported test may be caused by a number of factors: (a) The concurrent negative control rate reported by Seiler (57) is 0.28% PCEs with micronuclei; the historical and concurrent negative control rate experienced at SRI is 0.16%. This lower negative control rate results in a lower minimum incidence of micronuclei required to give a positive decision; (b) Different statistical methods were used. Seiler does not state what method was used, but his discussion implies that a comparison was made only with concurrent controls. The method used here assumes a negative binomial distribution and is based on historical control data; (c) Different protocols were used. Seiler does not report his method in detail, but refers to Schmid (36). The implication is that he dosed at 0 and 24 hours, with sacrifice at 30 hours; and (d) Seiler used an aqueous vehicle for oral dosing. DMSO can increase the absorption of chemicals over that experienced from aqueous solution.

Table 251

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D7

MONURON

			Survivors		Crossing-Over				Gene Conversion		Reverse Mutation	
					Mitotic Recombinants		Total Aberrants		Convertants		Revertants	
Compound	MA	% Conc.	Cells Per ml (× 10^{-7})	%	Per ml (× 10^{-3})	Per 10^5 Survivors	Per ml (× 10^{-3})	Per 10^5 Survivors	Per ml (× 10^{-1})	Per 10^6 Survivors	Per ml (× 10^0)	Per 10^7 Survivors
Negative Control												
DMSO	-	10	4.4	100	0	0	6.5	15	140	32	24	5.5
	+	10	4.4	100	2.0	4.5	14	32	150	34	19	4.3
Positive Control												
1,2,3,4-Diepoxybutane	-	0.013	4.0	91	68	170	650	1600	1200	300	580	150
	+	0.013	3.5	80	53	150	520	1500	1500	430	700	200
Monuron	-	0.05	3.7	84	1.0	2.7	15	41	170	46	13	3.5
	-	0.1	4.2	95	4.0	9.5	13	31	160	38	13	3.1
	-	0.5	3.7	84	0	0	14	38	130	35	12	3.2
	-	1.0	3.2	73	0	0	16	50	140	44	14	4.4
	-	2.5	3.7	84	0	0	13	35	100	27	11	3.0
	+	0.05	3.3	75	6.0	18	10	30	140	42	25	7.6
	+	0.1	3.8	86	3.0	7.9	10	26	150	39	27	7.1
	+	0.5	3.0	68	1.0	3.3	12	40	120	40	13	4.3
	+	1.0	3.4	77	1.0	2.9	12	35	110	32	17	5.0
	+	2.5	2.9	66	1.0	3.4	9.0	31	120	41	8.0	2.8

Table 252

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D7

MONURON

			Survivors		Crossing-Over				Gene Conversion		Reverse Mutation	
					Mitotic Recombinants		Total Aberrants		Convertants		Revertants	
Compound	MA	% Conc.	Cells Per ml ($\times 10^{-7}$)	%	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors	Per ml ($\times 10^{-1}$)	Per 10^6 Survivors	Per ml ($\times 10^0$)	Per 10^7 Survivors
Negative Control												
DMSO	-	10	5.2	100	0	0	12	23	43	8.3	22	4.2
	+	10	5.7	100	2.5	4.4	12	21	54	9.5	22	3.9
Positive Control												
1,2,3,4-Diepoxybutane	-	0.013	4.6	88	97	210	810	1800	1700	370	350	76
	+	0.013	6.3	111	100	160	870	1400	1600	250	310	49
Monuron	-	0.5	3.6	69	8.0	22	10	28	80	22	19	5.3
	-	1.0	3.5	67	7.0	20	18	51	52	15	28	8.0
	-	2.0	2.8	54	8.0	29	23	82	72	26	25	8.9
	-	3.0	3.2	62	5.0	16	18	56	93	29	18	5.6
	-	4.0	3.3	63	4.0	12	18	55	86	26	15	4.5
	+	0.5	3.0	53	4.0	13	10	33	66	22	19	6.3
	+	1.0	2.9	51	6.0	21	11	38	59	20	32	11
	+	2.0	3.9	68	4.0	10	12	31	76	19	14	3.6
	+	3.0	3.6	63	6.0	17	7.0	19	80	22	21	5.8
	+	4.0	4.2	74	6.0	14	18	43	81	19	21	5.0

Table 253

CYTOTOXICITY TEST OF CHINESE HAMSTER OVARY CELLS EXPOSED TO MONURON FOR 21.5 HOURS WITHOUT METABOLIC ACTIVATION

Treatment	Number of Divisions in Bromodeoxyuridine (BrdU)[*]
Negative control (1% DMSO)	$M_2 > M_1$[†]
Monuron (μg/ml)	
5.2	$M_2 >> M_1$[‡]
26	$M_1 > M_2$[†]
130	$M_1 > M_2$[†]
650	M_1
3250	M_1
Positive control (10^{-3} M ethyl methanesulfonate)	$M_2 > M_1$[†]

---

[*] M_1, one division in BrdU; M_2, two divisions in BrdU.

[†] The type of cell to left of > sign was in excess.

[‡] The type of cell to left of >> sign was in great excess.

Table 254

CYTOTOXICITY TEST OF CHINESE HAMSTER OVARY CELLS EXPOSED TO MONURON FOR 2 HOURS WITH METABOLIC ACTIVATION

Treatment	Number of Divisions in Bromodeoxyuridine (BrdU)*
Negative control (1% DMSO)	M_2
Monuron (µg/ml)	
4.04	M_2
20.2	$M_2 >> M_1$†
100.9	M_2
504.4	$M_2 >> M_1$†
2522	$M_1 >> M_2$†
Positive control (10^{-3} M dimethylnitrosamine)	M_2

---

*M_1, one division in BrdU; M_2, two divisions in BrdU.

†The type of cell to left of >> sign was in great excess.

Table 255

SISTER CHROMATID EXCHANGE (SCE) FREQUENCIES IN CHINESE HAMSTER OVARY (CHO) CELLS EXPOSED TO MONURON FOR 21.5 HOURS WITHOUT METABOLIC ACTIVATION

Treatment	Scorer*	No. of SCEs	No. of Chromosomes	Chromosomes/Cell (Mean ± SD)	SCEs/Cell† (Mean ± SE)	SCEs/ Chromosome† (Mean ± SE)
Negative control (1% DMSO)	A	318	486	19.44 ± 0.58	12.72 ± 0.71	0.654 ± 0.037
	B	286	502	20.08 ± 0.76	11.44 ± 0.68	0.570 ± 0.034
Monuron (μg/ml)						
0.625	A	320	483	19.32 ± 1.07	12.80 ± 0.72	0.663 ± 0.037
	B	290	505	20.20 ± 0.65	11.60 ± 0.68	0.574 ± 0.034
1.25	A	282	481	19.24 ± 0.66	11.28 ± 0.67	0.586 ± 0.035
	B	336	495	19.80 ± 1.08	13.44 ± 0.73	0.679 ± 0.037
2.5	A	301	485	19.40 ± 0.71	12.04 ± 0.69	0.621 ± 0.036
	B	327	496	19.84 ± 1.11	13.08 ± 0.72	0.659 ± 0.036
5	A	308	483	19.32 ± 0.75	12.32 ± 0.70	0.638 ± 0.036
	B	309	496	19.84 ± 0.62	12.36 ± 0.70	0.623 ± 0.035
10	A	319	479	19.16 ± 0.69	12.76 ± 0.71	0.666 ± 0.037
	B	303	491	19.64 ± 0.57	12.12 ± 0.70	0.617 ± 0.035
Positive control (10^{-3} M ethyl methanesulfonate)	A	1054	485	19.40 ± 0.76	42.16 ± 1.30	2.173 ± 0.067
	B	928	497	19.88 ± 0.93	39.28 ± 1.25	1.867 ± 0.063

---

[*] Each scorer analyzed 25 cells per sample.

[†] A one-way analysis of variance comparing the SCE frequencies in CHO cells exposed to monuron and to the negative control indicated that the variance between treatment groups was not significantly greater than the variance within treatment groups.

Table 256

SISTER CHROMATID EXCHANGE (SCE) FREQUENCIES IN CHINESE HAMSTER OVARY (CHO) CELLS EXPOSED TO MONURON FOR 2 HOURS WITH METABOLIC ACTIVATION

Treatment	Scorer*	No. of SCEs	No. of Chromosomes	Chromosomes/Cell (Mean ± SD)	SCEs/Cell† (Mean ± SE)	SCEs/ Chromosome† (Mean ± SE)
Negative control (1% DMSO)	A	247	456	18.24 ± 0.83	9.88 ± 0.63	0.542 ± 0.034
	B	326	483	19.32 ± 0.63	13.04 ± 0.72	0.675 ± 0.037
Monuron (µg/ml)						
125	A	396	458	18.32 ± 0.80	15.84 ± 0.80	0.846 ± 0.043
	B	444	481	19.24 ± 1.09	17.76 ± 0.84	0.923 ± 0.044
250	A	473	470	18.80 ± 1.12	18.92 ± 0.87	1.006 ± 0.046
	B	562	489	19.56 ± 0.77	22.48 ± 0.95	1.149 ± 0.049
500	A	522	471	18.84 ± 0.99	20.88 ± 0.91	1.108 ± 0.049
	B	689	482	19.28 ± 0.94	27.56 ± 1.05	1.430 ± 0.055
1000	A	646	470	18.80 ± 0.71	25.84 ± 1.02	1.375 ± 0.054
	B	655	486	19.44 ± 0.96	26.20 ± 1.02	1.348 ± 0.053
2000	A	535	462	18.48 ± 0.92	21.40 ± 0.93	1.158 ± 0.050
	B	665	476	19.04 ± 0.94	26.60 ± 1.03	1.397 ± 0.054
Positive control (10^{-3} M dimethylnitrosamine)	A	839	471	18.84 ± 1.03	33.56 ± 1.16	1.781 ± 0.061
	B	1014	478	19.16 ± 0.97	40.56 ± 1.27	2.157 ± 0.067

---

[*] Each scorer analyzed 25 cells per sample.

[†] A one-way analysis of variance comparing the SCE frequencies in CHO cells exposed in monuron and to the negative control indicated that the variance between treatment groups was significantly greater ($P < 0.025$ for SCEs/cell; $P < 0.01$ for SCEs/chromosome) than the variance within treatment groups.

Table 257

MOUSE LYMPHOMA FORWARD MUTATION CONCENTRATION-RANGE ASSAY OF MONURON IN THE ABSENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency (× 10^{-6})	Induced Mutation Frequency (× 10^{-6})
Solvent control (1% DMSO)	102.0	600	514	87	100.8	102.8	34	--
	98.0	600	506	102	99.2	97.2	40	--
Positive control (500 μg/ml EMS)	72.4	600	338	960	66.3	48.0	568	531
	72.4	600	263	977	55.5	40.2	690	653
Monuron								
1	97.5	600	432	122	84.7	82.6	56	19
2	102.2	600	483	108	94.7	96.8	45	8
5	97.3	600	--*	--*	--	--	--	--
10	107.7	600	538	119	105.5	113.6	44	7
20	97.3	600	--*	--*	--	--	--	--
40	102.2	600	573	157	112.4	114.8	55	18
80	97.3	600	514	108	100.8	98.1	42	5
100	97.3	600	474	127	92.9	90.4	54	17
150	88.9	600	511	121	100.2	89.1	47	10
200	81.8	600	489	122	95.9	78.4	50	13
300	72.6	600	529	116	103.7	75.3	44	7
400	65.3	600	517	122	101.4	66.2	47	10
500	44.0	600	521	129	102.2	44.9	50	13
600	34.1	600	495	161	97.1	33.1	65	28
700	26.4	600	472	229	92.5	24.4	97	60
800	25.0	600	487	223	95.5	23.9	92	55
900	PPT†	--	--	--	--	--	--	--
1000	PPT	--	--	--	--	--	--	--

---

*Sample lost.

†Persistent precipitate present after washdown procedure; sample discarded.

Table 258

MOUSE LYMPHOMA FORWARD MUTATION CONCENTRATION-RANGE ASSAY OF MONURON IN THE PRESENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency ($\times 10^{-6}$)	Induced Mutation Frequency ($\times 10^{-6}$)
Solvent control	105.3	600	582	231	99.8	105.1	79	--
(1% DMSO)	94.7	600	584	213	100.2	94.9	73	--
Positive control	61.6	600	336	578	57.6	35.5	344	268
(5 μg/ml 3-MCA)	60.6	600	404	571	69.3	42.0	283	207
Monuron								
1	109.2	600	567	244	97.3	106.2	86	10
2	109.2	600	526	248	90.2	98.5	94	18
5	93.1	600	498	282	85.4	79.5	113	37
10	80.2	600	554	339	95.0	76.2	122	46
20	64.7	600	433	398	74.3	48.1	184	108
40	19.7	600	350	329	60.0	11.8	188	112
80	2.1	NC*	--	--	--	--	--	--
100	1.2	NC	--	--	--	--	--	--
150	1.1	NC	--	--	--	--	--	--
200	1.3	NC	--	--	--	--	--	--
300	1.1	NC	--	--	--	--	--	--
400	1.2	NC	--	--	--	--	--	--
500	1.2	NC	--	--	--	--	--	--
600	1.2	NC	--	--	--	--	--	--
700	1.3	NC	--	--	--	--	--	--
800	1.2	NC	--	--	--	--	--	--
900	1.0	NC	--	--	--	--	--	--
1000	1.2	NC	--	--	--	--	--	--

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*Not cloned.

Table 259

MOUSE LYMPHOMA FORWARD MUTATION ASSAY OF MONURON IN THE ABSENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency ($\times 10^{-6}$)	Induced Mutation Frequency ($\times 10^{-6}$)
Solvent control	94.6	600	560	96	93.0	88.0	34	--
(1% DMSO)	105.4	600	644	97	107.0	112.8	30	--
Positive control	63.1	600	498	899	82.7	52.2	361	329
(500 μg/ml EMS)	68.7	600	424	926	70.4	48.4	437	405
Monuron								
50	108.4	600	519	81	86.2	93.5	31	--
	108.4	600	538	110	89.4	96.9	41	9
100	105.0	600	582	76	96.7	101.5	26	--
	101.0	600	532	93	88.4	89.3	35	3
308	82.8	600	560	144	93.0	77.0	51	19
	81.1	600	536	121	89.0	72.2	45	13
550	64.9	600	599	118	99.5	64.6	39	7
	65.6	600	596	145	99.0	64.9	49	17
748	50.3	500	482	197	80.1	40.3	82	50
	53.6	600	550	142	91.4	49.0	52	20
902	41.2	600	544	112	90.4	37.2	41	9
	45.3	600	565	124	93.9	42.5	44	12
1001	25.1	600	553	137	91.9	23.1	50	18
	33.1	600	510	222	84.7	28.0	87	55
1034	23.0	600	513	201	85.2	19.6	78	46
	22.3	600	555	133	92.2	20.6	48	16
1067	28.5	600	527	133	87.5	24.9	50	18
	28.7	600	551	229	91.5	26.3	83	51
1100	25.7	600	474	194	78.7	20.2	82	50
	37.8	600	584	174	97.0	36.7	60	28

Table 260

MOUSE LYMPHOMA FORWARD MUTATION ASSAY OF MONURON IN THE PRESENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency ($\times 10^{-6}$)	Induced Mutation Frequency ($\times 10^{-6}$)
Solvent control (1% DMSO)	105.7	600	560	211	100.6	106.4	75	--
	94.3	600	553	207	99.4	93.7	75	--
Positive control (6 μg/ml 3-MCA)	73.9	600	401	704	72.1	53.3	351	276
	68.6	600	415	732	74.6	51.2	353	278
Monuron								
6	87.9	600	541	312	97.2	85.5	115	40
	87.9	600	536	326	96.3	84.7	122	47
15	80.4	600	515	336	92.5	74.4	131	56
	83.1	600	578	416	103.9	86.3	144	69
21	75.3	600	592	427	106.4	80.1	144	69
	75.3	600	481	378	86.4	65.1	157	82
30	61.5	600	477	464	85.7	52.7	195	120
	67.6	600	490	448	88.1	59.5	183	108
36	49.7	600	498	450	89.5	44.5	181	106
	58.0	600	493	537	88.6	51.4	218	143
39	47.1	600	506	523	90.9	42.8	207	132
	57.2	600	515	486	92.5	52.9	189	114
42	44.5	600	454	520	81.6	36.3	229	154
	48.4	600	531	636	95.4	46.2	240	165
45	41.5	600	453	--*	81.4	33.8	--	--
	31.9	600	401	657	72.1	23.0	328	253
51	34.7	600	429	737	77.1	26.7	344	269
	41.2	600	426	669	76.5	31.5	314	239
60	27.7	600	391	656	70.3	19.5	336	261
	36.3	600	356	632	54.0	23.2	355	280

---

[*] Sample lost

Table 261

UNSCHEDULED DNA SYNTHESIS ASSAY OF MONURON WITHOUT METABOLIC ACTIVATION
(dpm/μg DNA)
Retest

Sample	Concentration of Compounds Tested						
	Monuron ($M \times 10^{-7}$)						
	0*	1.23	3.7	11.1	33.3	100	4NQO (M) (10^{-5})
1	68	56	58	71	70	137	1360
2	72	†	61	67	72	72	1237
3	75	70	55	96	67	74	1564
4	53	66	56	86	69	79	1095
5	49	71	74	94	63	87	1630
6	69	61	89	69	73	88	1381
Mean	64	64	65	81	69	90	1378
SD	11	6	13	13	4	24	199
SE	5	3	5	5	1	10	81

---

*Negative control and compound solvent, 0.5% DMSO.

†Sample lost.

Table 262

UNSCHEDULED DNA SYNTHESIS ASSAY OF MONURON WITH METABOLIC ACTIVATION

(dpm/μg DNA)

Retest 1

Sample	Concentration of Compounds Tested						
	Monuron (M x 10^{-5})						DMN (M)
	0*	3.7	11.1	33.3	100	300	5 x 10^{-2}
1	155	215	147	147	209	209	958
2	240	189	183	219	225	289	799
3	170	235	215	246	234	483	1070
4	187	198	258	206	212	212	598
5	225	205	180	225	119	176	905
6	234	255	268	149	158	161	452
Mean	202	216	209	199	193	255	797
SD	36	25	47	41	45	120	232
SE	15	10	19	17	18	49	95

---

*Negative control and compound solvent, 0.5% DMSO.

Table 263

UNSCHEDULED DNA SYNTHESIS ASSAY OF MONURON WITH METABOLIC ACTIVATION
(dpm/μg DNA)
Retest 2

	Concentration of Compounds Tested						
	Monuron ($M \times 10^{-4}$)						DMN (M)
Sample	0*	1.11	3.33	10	30	90	5×10^{-2}
1	108	93	110	94	112	78	461
2	130	92	121	83	100	87	249
3	83	98	117	94	140	76	278
4	106	83	83	95	72	93	230
5	101	103	93	85	76	75	204
6	117	102	101	89	69	154	248
Mean	108	95	104	90	95	94	278
SD	16	8	15	5	28	30	93
SE	6	3	6	2	11	12	38

---

*Negative control and compound solvent, 0.5% DMSO.

Table 264

DOSING AND SACRIFICE SCHEDULE

Compound	No. Animals	Dose		Mean Body Weight Change (g)	No. Sacrificed			No. Fatalities
		0 Hr	24 Hr		48 Hr	72 Hr	96 Hr	
DMSO--Vehicle Control	24	5 ml/kg	5 ml/kg	+ 0.4	6	7	7	4
Monuron								
Low Dose	24	500 mg/kg	500 mg/kg	+ 0.1	8	7	7	2
Medium Dose	24	1000 mg/kg	1000 mg/kg	- 0.2	8	8	8	0
High Dose	8	2000* mg/kg	2000† mg/kg	+ 0.1	3	-	3	2
	16	0	2000† mg/kg	-	4	8	3	1

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*This amount was not administered accurately because the compound was precipitating.

†in 10 ml/kg DMSO.

Table 265

INCIDENCE OF MICRONUCLEATED POLYCHROMATOPHILIC ERYTHROCYTES*
(500 PCEs Scored per Animal)

	48 Hours			72 Hours			96 Hours		
Dose	Animal No.	PCEs with MN	PCE/RBC	Animal No.	PCEs with MN	PCE/RBC	Animal No.	PCEs with MN	PCE/RBC
Vehicle**	20	1	0.99	10	1	1.36	30	0	0.72
Control	2r	1	0.93	1r	0	1.43	3r	1	0.70
DMSO	2ℓ	0	0.78	1ℓ	0	1.14	3ℓ	1	0.98
5 ml/kg	2b	0	1.41	1b	2	1.26	60	2	0.90
	5r	2	2.08	40	0	0.85	6r	1	1.98
	5ℓ	1	0.64	4r	1	1.69	6ℓ	1	1.31
				4b	2	1.47	6b	0	1.45
	Total	5			6			6	
	Mean		1.14			1.31			1.15
Monuron	270	2	1.64	250	2	0.65	260	2	2.13
2 x 500 mg/kg	27r	1	0.92	25r	1	1.19	26r	2	2.74
	27ℓ	0	1.27	25ℓ	2	1.53	26ℓ	1	2.53
	27b	1	1.90	25b	1	1.36	300	2	0.90
	280	4	0.78	290	2	1.64	30r	3	0.73
	28r	3	1.35	29r	1	1.36	30ℓ	3	0.70
	28ℓ	5	1.33	29b	1	1.30	30b	2	1.11
	28b	1	1.22						
	Total	17			10			15	
	Mean		1.30			1.29			1.55
Monuron	340	2	1.13	310	2	1.41	330	2	0.76
2 x 1000 mg/kg	34r	0	0.84	31r	1	1.06	33r	3	1.53
	34ℓ	3	1.57	31ℓ	0	1.72	33ℓ	0	0.79
	34b	1	0.84	31b	2	1.29	33b	1	1.48
	360	4	1.10	320	0	1.29	350	0	1.50
	36r	4	1.16	32r	2	1.26	35r	2	0.87
	36ℓ	0	1.16	32ℓ	2	1.96	35ℓ	0	1.04
	36b	2	1.44	32b	2	2.08	35b	0	1.15
	Total	16			11			8	
	Mean		1.16			1.51			1.14
Monuron	370	0	1.12	390	1	1.01	380	2	0.87
Cages 37 & 38	37r	0	0.93	39r	1	1.30	38r	0	1.30
2 x 2000 mg/kg	37ℓ	2	0.96	39ℓ	3	1.47	38ℓ	1	1.11
	400	4	1.04	39b	1	0.79	420	1	1.63
Cages 39-42	40r	1	1.05	410	1	0.79	42r	3	1.02
1 x 2000 mg/kg	40ℓ	3	1.00	41r	1	0.79	42b	2	1.09
at 24 hours	40b	2	0.95	41ℓ	1	0.73			
				41b	2	1.54			
	Total	12			11			9	
	Mean		1.01			1.05			1.17

---

* PCE = polychromatophilic erythrocytes; RBC = mature erythrocytes; MN = micronucleus.

**This is the same control reported with the micronucleus test on benomyl, which was performed simultaneously with this test.

MSMA

S. typhimurium Plate Incorporation (Table 266). MSMA was previously tested at concentrations up to 1000 μg/plate and found to be negative, with and without activation in strains TA100, TA1535, TA1537 and TA1538 (51). It was retested under the present contract at concentrations up to 5000 μg/plate and found to be negative with and without activation in strains TA98 and TA100.

E. coli WP-2, S. cerevisiae D3, E. coli/B. subtilis Relative Toxicity, UDS in WI-38 Cells. MSMA was previously tested in these systems and was found to be negative in all cases (51).

Table 266

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

MSMA
Retest

Compound	Metabolic Activation	Compound Added (µg/plate)	Histidine Revertants per Plate TA1535	TA1537	TA1538	TA98	TA100
Negative Control							
DMSO	-				25 16	28 30	135 138
	+				23 25	28 38	139 132
Positive Controls:							
2-Anthramine	-	1.0			52	42	145
	+	1.0			310	324	746
Sodium Azide	-	0.5					397
2-Nitrofluorene	-	5.0			817	543	
MSMA	-	500.0				25	120
	-	1,000.0				30	111
	-	2,000.0				17	126
	-	3,000.0				25	100
	-	4,000.0				25	114
	-	5,000.0				28	115
	+	500.0				30	125
	+	1,000.0				36	143
	+	2,000.0				35	121
	+	3,000.0				32	128
	+	4,000.0				43	114
	+	5,000.0				36	134

Parathion

S. typhimurium Plate Incorporation (Table 267). Parathion was previously tested at concentrations up to 1000 μg/plate in strains TA100, TA1535, TA1537 and TA1538 and found to be negative with and without activation (51). It was retested under the present contract at concentrations up to 5000 μg/plate in strains TA98 and TA100 and was negative with and without activation.

E. coli WP-2. This compound was found to be negative with and without activation in previous tests (51).

S. cerevisiae D3 (Table 268). Parathion was previously tested at one dose level and found to be negative (51). It was retested under the present contract over a range of doses, with similar findings.

E. coli/B. subtilis Relative Toxicity. This compound was previously tested and found to be negative in both species (51).

UDS in WI-38 Cells (Tables 269, 270). Parathion was previously tested and reported to be negative without activation, positive with activation (51). Since the latter response appeared somewhat weak, this compound was retested under the present contract and was found to be negative both with and without activation. We conclude that the latter interpretation is appropriate.

Mouse Dominant Lethal Test. Parathion was previously tested and found to be negative in this system (51).

Table 267

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

PARATHION

Retest

Compound	Metabolic Activation	Compound Added (μg/plate)	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative Control DMSO	-					24 16	135 110
	+					20 21	141 129
Positive Controls							
2-Anthramine	-	1.0				26	125
	+	1.0				232	527
Sodium Azide	-	0.5					286
2-Nitrofluorene	-	5.0				394	
Parathion	-	500.0				18	110
	-	1,000.0				20	93
	-	2,000.0				19	113
	-	3,000.0				13	101
	-	4,000.0				10	107
	-	5,000.0				10	108
	+	500.0				19	128
	+	1,000.0				18	115
	+	2,000.0				18	110
	+	3,000.0				23	121
	+	4,000.0				20	137
	+	5,000.0				17	136

Table 268

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

PARATHION

Retest

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml (x 10^{-7})	Percent	Per ml (x 10^{-3})	Per 10^3 Survivors
Negative Control: DMSO	-		8.0	100	4.0	5.0
	+		7.9	100	3.5	4.4
Positive Control 1,2,3,4-Diepoxybutane	-	0.025	7.8	98	902.5	1157.1
	+		C*	C	C	C
Parathion	-	1	4.7	59	6.0	12.7
	-	2	5.6	70	4.0	7.1
	-	3	C	C	C	C
	-	4	7.0		5.0	7.1
	-	5	7.5	95	4.0	5.3
	+	1	5.9	75	4.0	6.9
	+	2	7.9	100	7.0	8.9
	+	3	5.7	72	4.0	7.0
	+	4	5.9	75	6.0	10.2
	+	5	5.3	67	4.0	7.6

* C, contaminated.

Table 269

UNSCHEDULED DNA SYNTHESIS ASSAY OF PARATHION WITHOUT METABOLIC ACTIVATION

(dpm/μg DNA)

Retest

	Concentration of Compounds Tested						
	Parathion (M x 10^{-6})						4NQO (M)
Sample	0*	3.7	11.1	33.3	100	300	(10^{-5})
1	162	142	136	126	177	120	2293
2	158	154	202	149	150	119	2200
3	132	172	210	184	184	135	2604
4	109	192	157	132	188	139	2681
5	202	167	125	114	142	132	2066
6	160	142	148	131	137	166	2316
Mean	154	161	163	140	163	135	2360
SD	31	19	35	25	22	17	237
SE	13	8	14	10	9	7	97

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*Negative control and compound solvent, 0.5% DMSO.

Table 270

UNSCHEDULED DNA SYNTHESIS ASSAY OF ETHYL PARATHION WITH METABOLIC ACTIVATION
(dpm/μg DNA)
Retest

Sample	Concentration of Compounds Tested Ethyl-Parathion ($M \times 10^{-4}$) 0*	1.23	3.7	11.1	33.3	100	DMN (M) 5×10^{-2}
1	69	93	120	94	93	74	387
2	87	86	107	80	162	87	306
3	†	79	98	82	†	98	368
4	69	89	85	134	123	106	326
5	77	104	94	84	125	94	388
6	113	130	80	100	108	†	362
Mean	83	97	97	96	122	92	356
SD	18	18	15	20	26	12	34
SE	8	7	6	8	11	5	14

---

[*] Negative control and compound solvent, 0.5% DMSO.

[†] Sample lost.

Parathion-Methyl

S. typhimurium Plate Incorporation, E. coli WP-2. Parathion-methyl was previously tested and found to be negative, with and without activation in these two systems (51).

S. cerevisiae D3. This compound was previously tested and found to be positive with and without activation (51).

S. cerevisiae D7 (Tables 271-273). Parathion-methyl was first tested at concentrations ranging from 0.05 to 2.5% At a concentration of 1.0%, survival was reduced to below 10% and this concentration was considered toxic. In Experiments 2 and 3, concentrations ranged from 0.001 to 0.01% and 0.0025 to 0.02, respectively. The results from these three experiments indicate that no genetic damage was detected by the D7 strain.

E. coli/B. subtilis Relative Toxicity. This compound was previously tested and reported to be negative in both species (51). Reexamination of the data indicates that the results are more appropriately described as inconclusive.

S. typhimurium Relative Toxicity (Tables 274, 275). Parathion-methyl was negative in both strain pairs.

SCE in CHO Cells (Table 276-280). Parathion-methyl solution was dissolved in DMSO at a 50% concentration. At a concentration of 0.5%, parathion-methyl precipitated in the culture medium and in the metabolic activation mixture. Table 276 presents the results of the cytotoxicity test of parathion-methyl in CHO cell cultures without metabolic activation. At concentrations of 0.1% or greater, parathion-methyl was cytotoxic to CHO cells; at a concentration of 0.02%, the culture yielded primarily M_1 metaphases. At 0.004% and less, M_2 metaphases were produced. Table 275 presents the results of the cytotoxicity test of parathion-methyl in CHO cell cultures with metabolic activation. At concentrations of 0.1% or greater, parathion-methyl was cytotoxic to CHO cells; and at concentrations of 0.02% or less, primarily M_2 metaphases were produced.

Table 278 presents the SCE frequencies in CHO cells exposed to parathion-methyl without metabolic activation. Concentrations of 0.004% and greater produced insufficient M_2 metaphase cells for analysis. A one-way analysis of variance was conducted on SCE frequencies at the three lower concentrations of parathion-methyl and in the negative controls; the variance between treatment groups was not significantly greater than the variance within treatment groups. Thus, the compound was negative without activation.

Table 279 presents the SCE frequencies in CHO cells exposed to parathion-methyl with metabolic activation. Parathion-methyl at 0.04% was cytotoxic to the CHO cell cultures. A one-way analysis of variance was conducted on the SCE frequencies at the four lower concentrations of parathion-methyl and in the negative controls. The variance between treatment groups was not significantly greater than the variance within treatment groups. However, there was an indication of a concentration-related elevation in SCE frequencies in response to parathion-methyl.

A third cytogeneticist was asked to evaluate these coded slides. Table 280 presents the observations of the three scorers. The variance between treatment groups was significantly greater than the variance within treatment groups. The response appeared to be concentration-related, with the maximum (ranging from 1.2 to 2 times the negative control SCE frequencies, depending on the scorer) seen at 0.04%. Therefore, we conclude that CHO cells exposed to parathion-methyl with metabolic activation did show a small but statistically significant elevation in SCE frequencies.

L5178Y Mouse Lymphoma (Tables 281-284). Table 281 presents the results of concentration-range testing of parathion-methyl in the absence of the metabolic activation system. The results showed that the range to use in the mutagenesis assay was approximately 20 to 150 µg/ml (98.7% relative total growth at 20 µg/ml and 16.2% relative total growth at 150 µg/ml). Concentration-range test results for parathion-methyl in the presence of the metabolic activation system (Table 282) showed that the approximate range to use in the mutagenesis assay was 5 to 200 µg/ml (103.% relative total growth at 5 µg/ml and 3.0% relative total growth at 200 µg/ml).

Table 283 presents the results of mutagenesis testing of parathion-methyl in the presence of the metabolic activation system. The highest concentration (170 µg/ml) yielded an average relative total growth value of 12.8%. The lowest concentration (10 µg/ml) yielded an average total growth value of 84.0%. Between 10 and 170 µg/ml, a concentration-related increase in induced mutation frequency was observed. Average mutation frequencies of at least twice the spontaneous frequency were observed at 160 and 170 µg/ml (the two highest concentrations).

Table 284 presents the results of mutagenesis testing of parathion-methyl in the presence of the metabolic activation system. The highest concentration of 200 µg/ml yielded an average relative total growth value of 8.3%. No significant depression in relative total growth was observed at 50 µg/ml, which yielded an average relative total growth value of 92.3%. Over the tested range, a concentration-related increase in induced mutation frequency was observed. Average mutation frequencies at least twice the spontaneous frequency were observed from 80 to 176 µg/ml (seven concentrations), with average relative total growth values of over 10%.

These combined results indicate a mutagenic effect of parathion-methyl at the thymidine kinase locus in L5178Y mouse lymphoma cells, both with and without the metabolic activation system. In both cases, parathion-methyl increased the mutation frequency, in a concentration-related manner, above the spontaneous frequencies observed in the solvent controls. The presence of the metabolic activation system appeared to enhance the mutagenic effect of parathion-methyl.

UDS in WI-38 Cells. Parathion-methyl was previously tested and found to be negative with and without activation (51).

Mouse Dominant Lethal Test. This compound was previously tested in this system and found to be negative (51).

Table 271

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D7

PARATHION-METHYL

Experiment 1

			Survivors		Crossing-Over				Gene Conversion		Reverse Mutation	
					Mitotic Recombinants		Total Aberrants		Convertants		Revertants	
Compound	MA	% Conc.	Cells Per ml (× 10^{-7})	%	Per ml (× 10^{-3})	Per 10^5 Survivors	Per ml (× 10^{-3})	Per 10^5 Survivors	Per ml (× 10^{-1})	Per 10^6 Survivors	Per ml (× 10^0)	Per 10^7 Survivors
Negative Control												
DMSO	-	10	4.4	100	0	0	6.5	15	140	32	24	5.5
	+	10	4.4	100	2.0	4.5	14	32	150	34	19	4.3
Positive Control												
1,2,3,4-Diepoxybutane	-	0.013	4.0	91	68	170	650	1600	1200	300	580	150
	+	0.013	3.5	80	53	150	520	1500	1500	430	700	200
Parathion-Methyl	-	0.05	2.5	57	0	0	8.0	32	64	26	12	4.8
	-	0.1	1.5	34	2.0	13	7.0	47	64	43	3.0	2.0
	-	0.5	0.93	21	1.0	11	4.0	43	39	42	3.0	3.2
	-	1.0	T*		T		T		T		T	
	-	2.5	T		T		T		T		T	
	+	0.05	2.6	59	1.0	3.8	9.0	35	110	42	22	8.5
	+	0.1	2.2	50	2.0	9.1	12	55	100	45	13	5.9
	+	0.5	0.88	20	0	0	9.0	100	66	75	2.0	2.3
	+	1.0	T		T		T		T		T	
	+	2.5	T		T		T		T		T	

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*T = toxic

Table 272

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D7

PARATHION-METHYL

Experiment 2

			Survivors		Crossing-Over				Gene Conversion		Reverse Mutation	
					Mitotic Recombinants		Total Aberrants		Convertants		Revertants	
Compound	MA	% Conc.	Cells Per ml $(\times 10^{-7})$	%	Per ml $(\times 10^{-3})$	Per 10^5 Survivors	Per ml $(\times 10^{-3})$	Per 10^5 Survivors	Per ml $(\times 10^{-1})$	Per 10^6 Survivors	Per ml $(\times 10^0)$	Per 10^7 Survivors
Negative Control												
DMSO	-	10	7.7	100	1.0	1.3	13	17	80	10	18	2.3
	+	10	8.4	100	0	0	13	15	72	8.6	28	3.3
Positive Control												
1,2,3,4-Diepoxybutane	-	0.013	3.8	49	120	320	580	1500	1400	370	83	22
	+	0.013	6.3	75	93	150	600	950	2300	370	160	25
Parathion-Methyl	-	0.001	7.7	100	0	0	32	42	87	11	28	3.6
	-	0.0025	7.7	100	2.0	2.6	25	32	75	9.7	26	3.4
	-	0.005	8.2	107	2.0	2.4	28	34	94	11	29	3.5
	-	0.0075	7.1	92	0	0	22	31	92	13	24	3.4
	-	0.01	6.6	86	2.0	3.0	33	50	67	10	30	4.5
	+	0.001	8.0	95	0	0	24	30	75	9.4	24	3.0
	+	0.0025	8.5	101	0	0	20	24	88	10	23	2.7
	+	0.005	6.8	81	1.0	1.5	26	38	79	12	26	3.8
	+	0.0075	7.4	88	0	0	19	26	83	11	17	2.3
	+	0.01	6.8	81	0	0	24	35	72	11	28	4.1

Table 273

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D7

PARATHION-METHYL

Experiment 3

Compound	MA	% Conc.	Survivors		Crossing-Over				Gene Conversion		Reverse Mutation	
			Cells		Mitotic Recombinants		Total Aberrants		Convertants		Revertants	
			Per ml $(\times 10^{-7})$	%	Per ml $(\times 10^{-3})$	Per 10^5 Survivors	Per ml $(\times 10^{-3})$	Per 10^5 Survivors	Per ml $(\times 10^{-1})$	Per 10^6 Survivors	Per ml $(\times 10^{0})$	Per 10^7 Survivors
Negative Control												
DMSO	-	10	6.5	100	1.5	2.3	8.5	13	51	7.8	28	4.3
	+	10	7.7	100	0	0	9.5	12	70	9.1	25	3.2
Positive Control												
1,2,3,4-Diepoxybutane	-	0.013	5.8	89	40	69	400	690	1400	240	310	54
	+	0.013	6.9	90	54	78	460	670	1500	220	370	54
Parathion-Methyl	-	0.0025	9.0	138	0	0	11	12	70	7.8	34	3.8
	-	0.005	6.9	106	1.0	1.4	3.0	4.3	100	14	36	5.2
	-	0.0075	7.9	122	1.0	1.3	10	13	63	8.0	38	4.8
	-	0.01	8.2	125	3.0	3.7	12	15	82	10	34	4.1
	-	0.02	6.7	103	0	0	16	24	72	11	26	3.9
	+	0.0025	7.7	100	0	0	8.0	10	84	11	35	4.5
	+	0.005	8.0	104	0	0	10	13	70	8.8	35	4.4
	+	0.0075	7.9	103	0	0	6.0	7.6	89	11	46	5.8
	+	0.01	6.9	90	0	0	14	2.0	82	12	32	4.6
	+	0.02	6.6	86	1.0	1.5	10	15	67	10	31	4.7

Table 274

DIFFERENTIAL TOXICITY ASSAYS WITH SALMONELLA TYPHIMURIUM STRAINS

Experiment 1

Compound	Amount of Compound Applied to Disc	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		SL4525 (rec$^+$)		SL4700 (rec$^-$)		TA1978 (uvrB$^+$)		TA1538 (uvrB$^-$)	
Negative Control									
Kanamycin	100.0 µg	14	13	13	14	13	13	14	14
Positive Controls									
MMS*	10.0 mg	47	46	68	65				
4-NQO†	2.0 mg					12	12	24	23
Parathion-Methyl	5.0 µl	9	8	11	10	8	9	9	8
	10.0 µl	10	10	10	9	11	10	10	9

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* MMS = Methylmethanesulfonate.

† 4-NQO = 4-Nitroquinoline-1-oxide.

Table 275

DIFFERENTIAL TOXICITY ASSAYS WITH SALMONELLA TYPHIMURIUM STRAINS

Experiment 2

Compound	Amount of Compound Applied to Disc	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		SL4525 (rec$^+$)		SL4700 (rec$^-$)		TA1978 (uvrB$^+$)		TA1538 (uvrB$^-$)	
Negative Control									
Kanamycin	100.0 μg	15	14	16	15	17	17	15	16
Positive Controls									
MMS[*]	10.0 mg	45	40	72	74				
4-NQO[†]	2.0 mg					12	12	28	26
Parathion-Methyl	5.0 μl	8	7	8	8	10	9	8	8
	10.0 μl	7	8	8	8	11	8	9	8

---

* MMS = Methylmethanesulfonate.

† 4-NQO = 4-Nitroquinoline-1-oxide.

Table 276

CYTOTOXICITY TEST OF CHINESE HAMSTER OVARY CELLS EXPOSED TO PARATHION-METHYL FOR 21.5 HOURS WITHOUT METABOLIC ACTIVATION

Treatment	Number of Divisions in Bromodeoxyuridine (BrdU)[*]
Negative control (1% MSO)	M_2
Parathion-methyl solution (%)	
0.0008	M_2
0.004	$M_2 >> M_1$[†]
0.02	M_1
0.1	--[‡]
0.5[§]	--[‡]
Positive control (10^{-3} M ethyl methanesulfonate)	$M_2 > M_1$[**]

---

[*] M_1, one division in BrdU; M_2, two divisions in BrdU.

[†] The cell type to the left of the >> sign was in great excess.

[‡] These cultures were not harvested because of excessive cytotoxicity.

[§] Chemical precipitated in the culture medium.

[**] The cell type to the left of the > sign was in the majority.

Table 277

CYTOTOXICITY TEST OF CHINESE HAMSTER OVARY CELLS EXPOSED TO PARATHION-METHYL FOR 2 HOURS WITH METABOLIC ACTIVATION

Treatment	Number of Divisions in Bromodeoxyuridine (BrdU)*
Negative control (1% DMSO)	M_2
Parathion-methyl solution (%)	
0.0008	M_2
0.004	M_2 >> M_1 †
0.02	M_2
0.1	--‡
0.5§	--‡
Positive control (10^{-3} M dimethylnitrosamine)	M_2

---

[*] M_1, one division in BrdU; M_2, two divisions in BrdU.

[†] The cell type to the left of the >> sign was in great excess.

[‡] These cultures were not harvested because of excessive cytotoxicity.

[§] Chemical precipitated in the metabolic activation mixture.

Table 278

SISTER CHROMATID EXCHANGE (SCE) FREQUENCIES IN CHINESE HAMSTER OVARY (CHO) CELLS EXPOSED TO PARATHION-METHYL FOR 21.5 HOURS WITHOUT METABOLIC ACTIVATION

Treatment	Cyto- geneticist*	No. of SCEs	No. of Chromosomes	Chromosomes/Cell (Mean ± SD)	SCEs/Cell† (Mean ± SE)	SCEs/ Chromosomes† (Mean ± SE)
Negative control (1% DMSO)	A	321	478	19.12 ± 0.60	12.84 ± 0.72	0.672 ± 0.037
	B	294	481	19.24 ± 0.88	11.76 ± 0.69	0.611 ± 0.036
Parathion-methyl solution (%)						
0.0005	A	301	475	19.00 ± 0.91	12.04 ± 0.69	0.634 ± 0.017
	B	355	497	19.88 ± 0.67	14.20 ± 0.75	0.714 ± 0.038
0.001	A	384	476	19.04 ± 0.68	15.36 ± 0.79	0.807 ± 0.041
	B	322	484	19.36 ± 0.86	12.88 ± 0.72	0.665 ± 0.037
0.002	A	393	474	18.96 ± 0.89	15.72 ± 0.79	0.829 ± 0.042
	B	323	470	18.80 ± 1.08	12.92 ± 0.72	0.687 ± 0.038
0.004		--‡				
0.008		--‡				
Positive control (10^{-3} M ethyl methanesulfonate)	A	788	476	19.04 ± 0.74	31.52 ± 1.12	1.655 ± 0.059
	B	669	484	19.44 ± 0.65	26.76 ± 1.03	1.376 ± 0.053

---

[*] Each cytogeneticist analyzed 25 cells per sample.

[†] A one-way analysis of variance comparing the SCE frequencies in CHO cells exposed to parathion-methyl and to the negative control indicated that the variance between treatment groups was not significantly greater than the variance within treatment groups.

[‡] Insufficient M_2 metaphase cells for analysis.

Table 279

SISTER CHROMATID EXCHANGE (SCE) FREQUENCIES IN CHINESE HAMSTER OVARY (CHO) CELLS EXPOSED TO PARATHION-METHYL FOR 2 HOURS WITH METABOLIC ACTIVATION
(2 CYTOGENETICISTS)

Treatment	Cyto-geneticist*	No. of SCEs	No. of Chromosomes	Chromosomes/Cell (Mean ± SD)	SCEs/Cell[†] (Mean ± SE)	SCEs/Chromosome[†] (Mean ± SE)
Negative control (1% DMSO)	A	354	482	19.28 ± 0.74	14.16 ± 0.75	0.734 ± 0.039
	B	306	492	19.68 ± 0.95	12.24 ± 0.70	0.622 ± 0.036
Parathion-methyl solution (%)						
0.0025	A	358	488	19.52 ± 0.65	14.32 ± 0.76	0.734 ± 0.039
	B	320	485	19.40 ± 0.96	12.80 ± 0.72	0.660 ± 0.037
0.005	A	365	491	19.64 ± 0.64	14.60 ± 0.76	0.743 ± 0.039
	B	359	483	19.32 ± 1.03	14.36 ± 0.76	0.743 ± 0.039
0.01	A	391	482	19.28 ± 0.74	15.64 ± 0.79	0.811 ± 0.041
	B	339	497	19.88 ± 1.05	13.56 ± 0.74	0.682 ± 0.037
0.02	A	540	485	19.40 ± 0.50	21.60 ± 0.93	1.113 ± 0.048
	B	425	480	19.20 ± 0.76	17.00 ± 0.83	0.885 ± 0.043
0.04		--[‡]				
		--[‡]				
Positive control (10^{-3} M dimethylnitrosamine)	A	1059	495	19.80 ± 1.00	42.36 ± 1.30	2.139 ± 0.066
	B	979	507	20.28 ± 0.84	39.16 ± 1.25	1.931 ± 0.062

---

*Each cytogeneticist analyzed 25 cells per sample.

[†] A one-way analysis of variance comparing the SCE frequencies in CHO cells exposed to parathion-methyl and to the negative control indicated that the variance between treatment groups was not significantly greater than the variance within treatment groups.

[‡] Cytotoxic to CHO cell cultures.

Table 280

SISTER CHROMATID EXCHANGE (SCE) FREQUENCIES IN CHINESE HAMSTER OVARY (CHO) CELLS EXPOSED TO PARATHION-METHYL FOR 2 HOURS WITH METABOLIC ACTIVATION (3 CYTOGENETICISTS)

Treatment	Sample	Cyto-geneticist*	No. of SCEs	No. of Chromosomes	Chromosomes/cell (Mean ± SD)	SCEs/Cell† (Mean ± SE)	SCEs/Chromosome† (Mean ± SE)
Negative control (1% DMSO)	1	A	354	482	19.28 ± 0.74	14.16 ± 0.75	0.734 ± 0.039
	1	C	356	481	19.24 ± 0.97	14.24 ± 0.75	0.740 ± 0.039
	2	B	306	492	19.68 ± 0.95	12.24 ± 0.70	0.622 ± 0.036
Parathion-methyl solution (%)							
0.0025	3	B	320	485	19.40 ± 0.96	12.80 ± 0.72	0.660 ± 0.037
	4	A	358	488	19.52 ± 0.65	14.32 ± 0.76	0.734 ± 0.039
	4	C	343	476	19.04 ± 0.61	13.72 ± 0.74	0.721 ± 0.039
0.005	5	A	365	491	19.64 ± 0.64	14.60 ± 0.76	0.743 ± 0.039
	6	B	359	483	19.32 ± 1.03	14.36 ± 0.76	0.743 ± 0.039
	6	C	391	473	18.92 ± 1.12	15.64 ± 0.97	0.827 ± 0.042
0.01	7	A	391	482	19.28 ± 0.74	15.64 ± 0.79	0.811 ± 0.041
	8	B	339	497	19.88 ± 1.05	13.56 ± 0.74	0.682 ± 0.037
	8	C	420	480	19.20 ± 0.65	16.80 ± 0.82	0.875 ± 0.043
0.02	9	B	425	480	19.20 ± 0.76	17.00 ± 0.83	0.885 ± 0.043
	10	A	540	485	19.40 ± 0.50	21.60 ± 0.93	1.113 ± 0.048
	10	C	576	472	18.88 ± 0.93	23.04 ± 0.96	1.220 ± 0.051
0.04	11		--‡				
	12		--‡				
Positive control (10^{-3} M dimethylnitrosamine)	23	A	1059	495	19.80 ± 1.00	42.36 ± 1.30	2.139 ± 0.066
	24	B	979	507	20.28 ± 0.84	39.16 ± 1.25	1.931 ± 0.062

---

*Each cytogeneticist analyzed 25 cells per sample.

†A one-way analysis of variance comparing the SCE frequencies in CHO cells exposed to parathion-methyl and to the negative control indicated that the variance between treatment groups was significantly greater ($P < 0.005$) than the variance within treatment groups.

‡Cytotoxic to CHO cell cultures.

Table 281

MOUSE LYMPHOMA FORWARD MUTATION CONCENTRATION-RANGE ASSAY OF PARATHION-METHYL IN THE ABSENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency (x 10^{-6})	Induced Mutation Frequency (x 10^{-6})
Solvent control (1% DMSO)	104.7	600	541	98	99.8	104.5	36	--
	95.3	600	543	129	100.2	95.5	48	--
Positive control (500 μg/ml EMS)	66.7	600	332	990	61.3	40.9	596	554
	64.1	600	325	988	60.0	38.4	608	566
Parathion-methyl								
1	90.4	600	533	99	98.3	88.9	37	--
2	104.8	600	508	142	93.7	98.2	56	14
5	116.5	600	583	125	107.6	125.3	43	1
10	99.8	600	558	139	103.0	102.7	50	8
20	99.8	600	536	160	98.9	98.7	60	18
40	86.4	600	549	144	101.3	87.5	52	10
80	62.3	600	582	219	107.4	66.9	75	33
100	49.4	600	529	191	97.6	48.2	72	30
150	21.2	600	414	253	76.4	16.2	122	80
200	2.0	NC*	--	--	--	--	--	--
300	0.8	NC	--	--	--	--	--	--
400	PPT†	--	--	--	--	--	--	--
500	PPT	--	--	--	--	--	--	--
600	PPT	--	--	--	--	--	--	--
700	PPT	--	--	--	--	--	--	--
800	PPT	--	--	--	--	--	--	--
900	PPT	--	--	--	--	--	--	--
1000	PPT	--	--	--	--	--	--	--

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*Not cloned

†Persistent precipitate present after washdown procedure; sample discarded

Table 282

MOUSE LYMPHOMA FORWARD MUTATION CONCENTRATION-RANGE ASSAY OF PARATHION-METHYL IN THE PRESENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency ($\times 10^{-6}$)	Induced Mutation Frequency ($\times 10^{-6}$)
Solvent control	104.4	600	546	236	94.5	98.6	86	--
(1% DMSO)	95.6	600	610	249	105.5	100.9	82	--
Positive control	68.4	600	420	581	72.7	49.7	277	193
(5 μg/ml 3-MCA)	65.5	600	437	551	75.6	49.5	252	168
Parathion-methyl								
1	92.5	600	556	238	96.2	89.0	86	2
2	99.0	600	603	275	104.3	103.3	91	7
5	104.4	600	573	238	99.1	103.5	83	--
10	84.1	600	544	261	94.1	79.2	96	12
20	83.8	600	490	301	84.8	71.0	123	39
40	74.0	600	569	408	98.4	72.8	143	59
80	63.5	600	416	481	72.0	45.7	231	147
100	61.2	600	456	495	78.9	48.3	217	133
150	38.2	600	345	493	59.7	22.8	286	202
200	9.4	600	184	420	31.8	3.0	457	373
300	0.8	NC*	--	--	--	--	--	--
400	1.0	NC	--	--	--	--	--	--
500	1.0	NC	--	--	--	--	--	--
600	0.8	NC	--	--	--	--	--	--
700	0.7	NC	--	--	--	--	--	--
800	0.9	NC	--	--	--	--	--	--
900	PPT†	--	--	--	--	--	--	--
1000	PPT	--	--	--	--	--	--	--

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*Not cloned

†Persistent precipitate present after washdown procedure; sample discarded

Table 283

MOUSE LYMPHOMA FORWARD MUTATION ASSAY OF PARATHION-METHYL IN THE ABSENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency ($\times 10^{-6}$)	Induced Mutation Frequency ($\times 10^{-6}$)
Solvent control (1% DMSO)	99.5	600	626	154	98.0	97.5	49	--
	100.5	600	652	176	102.0	102.5	54	--
Positive control (500 μg/ml EMS)	71.0	600	298	1243	46.6	33.1	834	782
	67.5	600	310	1199	48.5	32.7	774	722
Parathion-methyl								
10	108.4	600	515	182	80.6	87.4	71	19
	103.2	600	499	161	78.1	80.6	65	13
50	103.6	600	458	169	71.7	74.3	74	22
	98.3	600	555	186	86.9	85.4	67	15
80	91.9	600	581	218	90.9	83.6	75	23
	88.4	600	627	202	98.1	86.7	64	12
100	85.2	600	623	234	97.5	83.1	75	23
	89.8	600	629	241	98.4	88.4	77	25
120	70.8	600	636	233	99.5	70.5	73	21
	76.9	600	641	231	100.3	77.1	72	20
130	69.9	600	614	203	96.1	67.2	66	14
	72.2	600	550	215	86.1	62.1	78	26
140	66.1	600	506	250	79.2	52.3	99	47
	66.1	600	589	191	92.2	60.9	65	13
150	44.2	600	597	327	93.4	41.3	79	27
	54.3	600	544	219	85.1	46.2	81	29
160	32.7	600	535	335	83.7	27.4	125	73
	39.4	600	472	290	73.9	29.1	123	71
170	18.3	600	543	371	85.0	15.6	137	85
	13.0	600	490	292	76.7	10.0	119	67

Table 284

MOUSE LYMPHOMA FORWARD MUTATION ASSAY OF PARATHION-METHYL IN THE PRESENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency (× 10^{-6})	Induced Mutation Frequency (× 10^{-6})
Solvent control (1% DMSO)	99.3	600	646	230	102.7	102.0	71	--
	100.7	600	612	246	97.3	98.0	80	--
Positive control (6 μg/ml 3-MCA)	76.1	600	466	665	74.1	56.4	285	209
	74.5	600	458	694	72.8	54.2	303	227
Parathion-methyl								
10	108.8	600	582	222	92.5	100.7	76	--
	96.2	600	544	228	86.5	83.2	84	8
50	96.1	600	669	370	106.4	102.2	111	35
	88.0	600	589	420	93.6	82.4	143	67
80	82.6	600	541	517	86.0	71.0	191	115
	82.6	600	628	640	99.8	82.5	204	128
100	73.9	600	461	741	73.3	54.2	321	245
	88.0	600	524	717	83.3	73.3	274	198
130	63.0	600	450	882	71.5	45.1	392	316
	65.9	600	406	841	64.5	42.5	414	338
150	55.1	600	378	754	60.1	33.1	399	323
	56.0	600	403	717	64.1	35.9	356	280
160	47.6	600	371	728	59.0	28.1	392	316
	44.7	600	303	776	48.2	21.5	512	436
166	41.8	600	320	748	50.9	21.3	468	392
	40.5	600	300	805	47.7	19.3	537	461
176	36.8	600	311	761	49.4	18.2	489	413
	38.0	600	253	800	40.2	15.3	632	556
200	22.3	600	230	756	36.6	8.2	657	581
	25.4	600	208	676	33.1	8.4	650	574

Pentachlorophenol

S. typhimurium Plate Incorporation, E. coli WP-2 (Tables 285, 286). Pentachlorophenol was tested twice in duplicate, on separate days, over a dose range of 1 to 100 µg/plate. No reproducible dose-related increase in the number of revertants was observed in either test system. In the *S. typhimurium* test, a toxic response, denoted by the appearance of pinpoint colonies and/or a thinning of the background lawn, was observed at 100 µg/plate with TA1537 and TA100 in the presence and absence of metabolic activation and with TA1538 without activation.

S. cerevisiae D3 (Tables 287, 288). Pentachlorophenol was tested twice on separate days over concentrations ranging from 0.001 to 0.05%. It caused a reproducible, dose-related increase in the number of mitotic recombinants per 10^5 survivors in the presence and in the absence of the rat liver metabolic activation system.

E. coli/B. subtilis Relative Toxicity (Table 289). Sonification was used to dissolve the compound in DMSO, but the highest pentachlorophenol dose tested (5 mg in 10 µℓ) precipitated. In three experiments, pentachlorophenol was reproducibly negative in the *E. coli* test. On the other hand, pentachlorophenol was more toxic to the repair-deficient (*rec*$^-$) than to the repair-proficient (*rec*$^+$) *B. subtilis* strain. This positive result indicates that pentachlorophenol causes DNA damage that is ameliorated by the *rec* repair capability.

Table 285

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

PENTACHLOROPHENOL

Experiment 1

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate										Tryptophan Revertants per Plate	
			TA1535		TA1537		TA1538		TA98		TA100		WP2	
Negative control (DMSO)	-		14	21	10	7	10	7	17	18	89	86	31	36
	+		5	11	10	12	17	26	28	29	87	84	48	37
Positive controls														
Sodium azide	-	1.0	474	430							557	549		
9-Aminoacridine	-	50.0			207	272								
2-Nitrofluorene	-	5.0					1118	1024	532	638				
AF2	-	0.1											990	1130
2-Anthramine	-	1.0					15	14	20	18	102	95		
	+	1.0					265	252	257	284	475	501		
	-	2.5	19	22	7	6								
	+	2.5	256	13	63	75								
	-	10.0											33	38
	+	10.0											989	1154
Pentachlorophenol	-	1	19	16	11	8	15	15	21	26	72	66	50	39
	-	5	33	26	7	10	9	9	17	20	80	78	41	50
	-	10	23	25	7	8	10	11	27	15	78	98	50	45
	-	25	14	15	10	9	16	11	15	28	65	87	45	48
	-	50	17	15	5	3	8	8	25	18	51	53	37	38
	-	100	22	11	Toxic		Toxic		13	13	Toxic		48	41
	+	1	10	5	9	16	26	13	29	34	98	78	41	49
	+	5	16	14	11	15	27	25	26	30	99	97	52	52
	+	10	15	12	8	16	28	18	28	40	100	92	63	56
	+	25	11	5	9	18	30	27	33	31	104	56	43	44
	+	50	9	6	8	2	23	22	26	29	99	76	37	39
	+	100	2	9	Toxic		9	7	20	23	Toxic		36	45

Table 286

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

PENTACHLOROPHENOL

Experiment 2

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate										Tryptophan Revertants per Plate	
			TA1535		TA1537		TA1538		TA98		TA100		WP2	
Negative control (DMSO)	-		37	37	17	6	15	7	29	33	127	122	29	44
	+		8	19	8	14	19	26	42	49	126	111	54	50
Positive controls														
Sodium azide	-	0.5	356											
	-	1.0									537			
9-Aminoacridine	-	50.0			506									
2-Nitrofluorene	-	5.0					872		489					
AF2	-	0.1											1927	1988
2-Anthramine	-	1.0					14		30		120			
	+	1.0					238		262		239			
	-	2.5	28		9									
	+	2.5	86		49									
	-	10.0											44	55
	+	10.0											480	474
Pentachlorophenol	-	1	32	32	8	5	12	16	30	23	130	146	30	38
	-	5	34	32	6	7	12	10	18	22	148	147	29	26
	-	10	30	38	10	11	10	14	20	23	142	140	37	36
	-	25	35	28	11	7	15	16	20	16	151	126	39	37
	-	50	30	31	4	6	18	15	22	14	158	140	46	38
	-	100	30	22	Toxic		8	8	21	14	126	129	30	25
	+	1	16	25	18	17	28	26	35	33	141	C*	39	41
	+	5	13	20	23	15	22	30	24	37	130	127	41	43
	+	10	19	18	18	24	18	26	35	37	148	140	45	49
	+	25	14	12	17	16	21	28	31	36	146	131	43	39
	+	50	18	16	6	16	21	22	34	32	153	126	38	50
	+	100	12	9	Toxic		12	9	33	17	127	135	39	38

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*C, contaminated.

Table 287

IN VITRO ASSAYS WITH *SACCHAROMYCES CEREVISIAE* D3

Pentachlorophenol

Experiment 1

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($x\ 10^{-7}$)	Percent	Per ml ($x\ 10^{-3}$)	Per 10^5 Survivors
Negative control (DMSO)	-		4.7	100	4.0	8.5
	+		4.4	100	1.5	3.4
Positive control 1,2,3,4-Diepoxybutane	-	0.025	4.7	100	423	900
	+	0.025	4.5	102	418	930
Pentachlorophenol	-	0.001	5.5	117	5.0	9.1
	-	0.005	4.3	91	7.0	16
	-	0.01	3.4	72	7.0	21
	-	0.02	1.8	38	8.0	44
	-	0.05	0.4	9	3.0	75
	+	0.001	6.9	157	5.0	7.3
	+	0.005	4.7	107	7.0	15
	+	0.01	4.4	100	7.0	16
	+	0.02	2.8	64	11.0	39
	+	0.05	1.0	23	13.0	130

Table 288

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

Pentachlorophenol

Experiment 2

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($x\ 10^{-7}$)	Percent	Per ml ($x\ 10^{-3}$)	Per 10^5 Survivors
Negative control (DMSO)	-		4.7	100	3.5	7.5
	+		6.1	100	1.5	2.5
Positive control 1,2,3,4-Diepoxybutane	-	0.025	4.9	104	913	1900
	+	0.025	4.2	69	723	1700
Pentachlorophenol	-	0.001	5.6	119	23.0	41
	-	0.005	3.5	74	18.0	51
	-	0.01	2.0	43	18.0	90
	-	0.02	0.8	17	15.0	190
	-	0.05	Toxic			
	+	0.001	3.4	56	11.0	32
	+	0.005	2.8	46	18.0	64
	+	0.01	2.0	33	14.0	70
	+	0.02	1.6	26	19.0	120
	+	0.05	Toxic			

Table 289

DIFFERENTIAL TOXICITY ASSAYS
PENTACHLOROPHENOL

Compound	Amount	Zone of Inhibition of Growth (in mm, disc=6 mm)							
		E. coli				B. subtilis			
		W3110 (polA$^+$)		p3478 (polA$^-$)		H17 (rec$^+$)		M45 (rec$^-$)	
EXPERIMENT 1									
Negative control Ampicillin	0.01 mg	20	20	18	17	25	26	28	27
Positive control Methylmethane sulfonate	1.0 mg	30	30	35	35	14	14	46	49
Pentachlorophenol	1.0 mg	16	16	11	12	30	30	31	33
	5.0	16	16	11	12	32	30	31	33
EXPERIMENT 2									
Negative control Ampicillin	0.01 mg	19	18	17	17	25	25	27	27
Positive control Methylmethane sulfonate	1.0 mg	16	16	22	23	15	15	50	50
Pentachlorophenol	1.0 mg	13	13	10	9	32	32	39	38
	5.0	12	13	10	11	36	39	38	38

(Continued)

Table 289 (Concluded)

Compound	Amount	Zone of Inhibition of Growth (in mm, disc=6 mm)							
		E. coli				B. subtilis			
		W3110 ($polA^+$)		p3478 ($polA^-$)		H17 (rec^+)		M45 (rec^-)	
EXPERIMENT 3									
Negative control Ampicillin	0.01 mg	18	19	18	18	24	24	28	25
Positive control Methylmethane sulfonate	1.0 mg	12	12	29	27	12	12	46	47
Pentachlorophenol	0.01 mg	9	10	9	9	18	20	24	24
	0.05	13	13	10	10	23	24	32	33
	0.5	15	15	11	10	28	27	37	37
	1.0	16	15	11	10	28	27	37	38
EXPERIMENT 4									
Negative control Kanamycin	0.10 mg	NT*		NT		16	16	17	16
Positive control Methylmethane sulfonate	10.0 mg	NT		NT		34	34	70	69
Pentachlorophenol	0.01 mg	NT		NT		17	16	20	19
	0.05	NT		NT		25	24	29	30
	0.5	NT		NT		25	28	32	33

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*NT, not tested.

Phorate

S. typhimurium Plate Incorporation (Table 290). Phorate was previously tested and found negative, with and without activation, in strains TA100, TA1535, TA1537 and TA1538 at concentrations up to 1000 µg/plate (51). Under the present contract it was retested at concentrations up to 5000 µg/plate in strains TA98 and TA100, and was found negative with and without activation.

E. coli WP-2, S. cerevisiae D3, E. coli/B. subtilis Relative Toxicity, UDS in WI-38 Cells, Mouse Dominant Lethal Test. This compound was previously tested in these systems and was found to be negative in all cases (51).

Table 290

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

PHORATE

Retest

Compound	Metabolic Activation	Compound Added (µg/plate)	Histidine Revertants per Plate TA1535	TA1537	TA1538	TA98	TA100
Negative Control							
DMSO	-					24 16	135 110
	+					20 21	141 129
Positive Controls							
2-Anthramine	-	1.0				26	125
	+	1.0				232	527
Sodium Azide	-	0.5					286
2-Nitrofluorene	-	5.0				394	
Phorate	-	500.0				11	92
	-	1,000.0				11	77
	-	2,000.0				12	76
	-	3,000.0				16	93
	-	4,000.0				14	79
	-	5,000.0				17	73
	+	500.0				25	110
	+	1,000.0				16	96
	+	2,000.0				23	97
	+	3,000.0				21	109
	+	4,000.0				21	112
	+	5,000.0				20	105

Propanil

S. typhimurium Plate Incorporation, E. coli WP-2, S. cerevisiae D3. Propanil was previously tested in these three systems and was found to be negative in all cases (52).

E. coli/B. subtilis Relative Toxicity. This compound was previously tested and reported as positive in both species with and without activation (52). Reexamination of the data indicates that it is more appropriately described as negative in E. coli, positive in B. subtilis.

S. typhimurium Relative Toxicity (Tables 291, 292). This compound was positive in the uvrB pair, but negative in the rec pair.

UDS in WI-38 Cells. Propanil was previously tested and found to be negative with and without activation (52).

Table 291

DIFFERENTIAL TOXICITY ASSAYS WITH *SALMONELLA TYPHIMURIUM* STRAINS

Experiment 1

Compound	Amount of Compound Applied to Disc	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		SL4525 (rec$^+$)		SL4700 (rec$^-$)		TA1978 (uvrB$^+$)		TA1538 (uvrB$^-$)	
Negative Control									
Kanamycin	100.0 µg	14	13	13	14	13	13	14	14
Positive Controls									
MMS*	10.0 mg	47	46	68	65				
4-NQO†	2.0 mg					12	12	24	23
Propanil	1.0 mg	11	13	13	14	13	13	16	16
	5.0 mg	13	13	15	14	13	13	15	16

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* MMS = Methylmethanesulfonate.

† 4-NQO = 4-Nitroquinoline-1-oxide.

Table 292

DIFFERENTIAL TOXICITY ASSAYS WITH SALMONELLA TYPHIMURIUM STRAINS

Experiment 2

Compound	Amount of Compound Applied to Disc	Zone of Inhibition of Growth (in mm, disc = 6 mm) SL4525 (rec$^+$)		SL4700 (rec$^-$)		TA1978 (uvrB$^+$)		TA1538 (uvrB$^-$)	
Negative Control									
Kanamycin	100.0 µg	15	14	16	15	17	17	15	16
Positive Controls									
MMS*	10.0 mg	45	40	72	74				
4-NQO†	2.0 mg					12	12	28	26
Propanil	1.0 mg	12	14	16	16	9	9	20	19
	5.0 mg	12	11	12	14	9	10	18	20

---

* MMS = Methylmethanesulfonate.

† 4-NQO = 4-Nitroquinoline-1-oxide.

Rotenone

Micronucleus Test (Tables 293, 294). The dosing and sacrifice schedules are shown in Table 293. The incidences of micronuclei in PCEs and the PCE-to-RBC ratios are given in Table 294. Rotenone was negative at all dose levels and sampling times, with a β error of < 0.005 in all groups. The DMSO vehicle control group was negative at all times, and the TMP positive control group was positive ($\alpha < 0.001$). The mean PCE-to-RBC ratios were within normal limits (0.50-1.50) for all groups except the low-dose, 96-hour rotenone group, which was elevated because of an aberrant value for one animal. Rotenone meets all of the criteria for a nonmutagenic decision in the micronucleus test.

Table 293

DOSING AND SACRIFICE SCHEDULE

Compound	No. of Animals	Dose		Mean Body Wt Change (g)	Number Sacrificed			No. of Fatalities
		0 Hr	24 Hr		48 Hr	72 Hr	96 Hr	
DMSO								
Vehicle control	24*	5 ml/kg	5 ml/kg	-1.2	8	7	8	1
TMP								
Positive control	8*	1 g/kg	1 g/kg	-2.4	8	-	-	0
Rotenone								
Low dose	24	0.56 mg/kg	0.56 mg/kg	-1.3	7	8	8	1
Mid dose	24	1.13 mg/kg	1.13 mg/kg	-1.0	8	8	8	0†
High dose	24	2.25 mg/kg	2.25 mg/kg	-1.0	8	8	7	1

---

* The control groups are the same as those reported with trichlorophon, which was treated at the same time.

† One animal sacrificed at 72 hr was cyanotic and moribund.

Table 294

INCIDENCE OF MICRONUCLEATED POLYCHROMATOPHILIC ERYTHROCYTES*

(500 PCE scored per animal)

	48 Hours			72 Hours			96 Hours		
Group	Animal No.	PCE with MN	PCE/RBC	Animal No.	PCE with MN	PCE/RBC	Animal No.	PCE with MN	PCE/RBC
DMSO 2 x 5 ml/kg Vehicle Control	101ø	1	1.30	103ø	1	1.98	102ø	0	0.70
	101r	0	1.49	103r	1	1.83	102r	1	1.00
	101ℓ	2	1.09	103ℓ	0	1.61	102ℓ	0	1.09
	101b	0	0.88	103b	2	0.59	102b	0	0.81
	104ø	1	0.81	106r	1	1.28	105ø	1	0.98
	104r	1	0.75	106ℓ	1	1.47	105r	1	1.56
	104ℓ	1	0.92	106b	1	1.23	105ℓ	1	1.24
	104b	0	1.47				105b	0	1.67
Total		6†			7§			4†	
Mean			1.09			1.43			1.13
Rotenone 2 x 0.56 mg/kg	112ø	0	1.44	111ø	0	0.93	110ø	0	1.45
	112r	1	1.79	111r	0	1.08	110r	1	1.62
	112ℓ	0	0.73	111ℓ	0	1.26	110ℓ	1	1.74
	115ø	0	1.17	111b	0	1.45	110b	0	0.93
	115r	0	1.18	114ø	0	1.01	113ø	0	2.94
	115ℓ	0	1.18	114r	0	1.76	113r	1	1.77
	115b	0	1.09	114ℓ	0	0.92	113ℓ	0	1.28
				114b	1	0.73	113b	0	1.44
Total		1†			1†			3†	
Mean			1.23			1.14			1.65
Rotenone 2 x 1.13 mg/kg	117ø	0	1.14	116ø	1	0.98	118ø	0	1.26
	117r	0	1.09	116r	0	1.26	118r	0	0.87
	117ℓ	0	1.09	116ℓ	1	1.30	118ℓ	1	1.58
	117b	1	0.66	116b	1	1.27	118b	0	0.74
	119ø	0	0.92	120ø	0	0.10	121ø	1	0.84
	119r	0	1.80	120r	0	0.81	121r	1	2.27
	119ℓ	1	0.99	120ℓ	0	2.51	121ℓ	1	1.13
	119b	0	1.37	120b	1	0.60	121b	2	0.90
Total		2†			4†			6†	
Mean			1.13			1.10			1.20

---

* PCE = Polychromatophilic erythrocytes, RBC = mature erythrocytes, MN = micronucleus.

† $\beta \leq 0.001$.

§ $\beta \leq 0.01$.

Table 294 (Concluded)

Group	48 Hours			72 Hours			96 Hours		
	Animal No.	PCE with MN	PCE / RBC	Animal No.	PCE with MN	PCE / RBC	Animal No.	PCE with MN	PCE / RBC
Rotenone 2 x 2.25 mg/kg	125ø	0	1.40	122ø	0	1.35	124ø	0	1.00
	125r	1	1.28	122r	0	1.63	124	1	0.64
	125ℓ	1	1.90	122ℓ	0	1.26	124b	1	1.59
	125b	0	1.18	122b	1	1.08	126ø	0	1.64
	127ø	0	1.40	123ø	0	0.91	126r	2	1.59
	127r	2	1.34	123r	2	1.41	126ℓ	1	1.20
	127ℓ	1	1.30	123ℓ	0	1.11	126b	0	0.83
	127b	2	1.16	123b	0	0.78			
Total		7‡			3†			5‡	
Mean			1.37			1.19			1.21
TMP 2 x 1 g/kg Positive Control	107ø	4	0.92						
	107r	18	0.62						
	107ℓ	2	0.92						
	107b	5	1.51						
	108ø	3	0.71						
	108r	4	1.32						
	108ℓ	0	1.01						
	108b	2	0.90						
Total		38**							
Mean			0.99						

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† $\beta \leq 0.001$.
‡ $\beta \leq 0.005$.
** $\alpha \leq 0.001$.

Simazine

S. typhimurium Plate Incorporation (Table 295). Simazine was previously tested and found to be negative with and without activation at concentrations up to 1000 μg/plate in strains TA100, TA1535, TA1537, and TA1538 (51). Under the present contract it was retested and found to be negative with and without activation in strains TA98 and TA100 at concentrations up to 5000 μg/plate.

E. coli WP-2, S. cerevisiae D3. This compound was previously tested and found to be negative with and without activation in these two test systems (51).

S. cerevisiae D7 (Tables 296, 297). Simazine was tested twice over concentrations ranging from 0.05 to 2.5%. The solubility of this compound at higher concentrations precluded the testing of doses greater than 2.5%. No increase in any of the three genetic end points was observed.

E. coli/B. subtilis Relative Toxicity. This compound was previously tested and reported to be negative in both species (51). Reexamination of the data indicates that the results are more appropriately described as inconclusive.

S. typhimurium Relative Toxicity (Tables 298, 299). Simazine was negative in both strain pairs.

SCE in CHO Cells (Tables 300-303). Simazine was dissolved in DMSO at a concentration of 173.83 mg/ml for cytotoxicity testing with metabolic activation and at a concentration of 154.9 mg/ml for cytotoxicity testing without metabolic activation. This difference in the solubility of simazine in DMSO may have been caused by a difference in temperatures in the laboratory on the days the experiments were performed.

In the cytotoxicity test without metabolic activation, simazine precipitated in culture medium at concentrations of 309.8 μg/ml and higher. In the cytotoxicity test with metabolic activation, the culture medium foamed when simazine was added at a concentration of 1738.3 μg/ml. In the SCE test with metabolic activation no precipitation was observed, while in the SCE test without metabolic activation, simazine precipitated at concentrations of 212.5 μg/ml and higher.

Table 300 presents the results of the cytotoxicity test of simazine in CHO cell cultures without metabolic activation. At all concentrations tested, M_2 metaphase cells were produced. Table 301 presents the results of the cytotoxicity test of simazine in CHO cell cultures with metabolic activation. At all concentrations tested, the cultures yielded M_2 metaphase cells.

Table 302 presents the SCE frequencies in CHO cells exposed to simazine without metabolic activation. One sample exposed to 1700 μg/ml simazine could not be analyzed because of poor contrast of the prepared slides, making them unscorable. A one-way analysis of variance was conducted on SCE frequencies of the other four concentrations of simazine and of the negative controls. The variance between treatment groups was not significantly greater than the variance within treatment groups.

Table 303 presents the SCE frequencies in CHO cells exposed to simazine with metabolic activation. A one-way analysis of variance was conducted on SCE frequencies of the five concentrations of simazine and of the negative controls. The variance between treatment groups was not significantly greater than the variance within treatment groups.

We conclude that simazine did not induce SCEs in CHO cells, either with or without metabolic activation.

L5178Y Mouse Lymphoma (Tables 304-307). Table 304 presents the results of concentration-range testing of simazine in the absence of the metabolic activation system. The results showed that the approximate range to use in the mutagenesis assay was 20 µg/ml to a concentration greater than 100 µg/ml (the highest concentration that was soluble in the cell suspension). The relative total growth values at 20 and 100 µg/ml were 97.9% and 80.1%, respectively.

Concentration-range tests for simazine in the presence of the metabolic activation system (Table 305) showed that the approximate range to use in the mutagenesis assay was 5 µg/ml to 300 µg/ml (the highest concentration that was soluble in the cell suspension). The relative total growth values at 5 and 300 µg/ml were 97.2% and 36.9%, respectively.

Table 306 presents the results of mutagenesis testing of simazine in the absence of the metabolic activation system. The solubility of simazine in the cell suspension was higher than that achieved in the concentration-range assay because the stock solution used for the high doses was less concentrated than that used in the concentration-range assay. However, even at the highest concentration (400 µg/ml), the average relative total growth was 102.0%. No cytotoxicity was observed at any of the concentrations tested. Solubility experiments confirmed that 400 µg/ml was the maximum solubility of simazine in the cell suspension. In the concentration range tested, there were no concentrations that yielded an average mutation frequency of at least twice the spontaneous frequency, and there was no evidence of a concentration-related increase in the induced mutation frequency.

Table 307 presents the results of mutagenesis testing in the presence of the metabolic activation system. At 300 µg/ml the average relative total growth value was 69.9%. No significant depression in relative total growth was observed at 20 µg/ml, which yielded an average relative total growth value of 93.9%. Over the range tested, a concentration-related increase in induced mutation frequency was observed. An average mutation frequency of at least twice the spontaneous frequency was observed at 300 µg/ml. For the present we interpret these results as positive, since they meet the specified criteria. However, additional testing to confirm this interpretation appears desirable.

These combined results indicate a mutagenic effect of simazine at the thymidine kinase locus in L5178Y mouse lymphoma cells in the presence of the metabolic activation system. In the absence of the metabolic activation system, insufficient solubility of simazine in the cell suspension precluded testing to cytotoxic levels; hence the results are inconclusive. With metabolic activation, simazine increased the mutation frequency, in a concentration-related manner, above the frequencies observed with the solvent control.

UDS in WI-38 Cells. Simazine was previously tested and found to be negative, both with and without activation (51).

Micronucleus Test (Tables 308, 309). The dosing and sacrifice schedules are shown in Table 308. The incidences of micronucleated PCEs to RBCs are given in Table 309. The results for all negative control and simazine-treated groups were negative ($\beta < 0.05$) except as discussed below. The TMP-treated groups were positive ($\alpha < 0.001$). The mean ratios of PCEs to RBCs were within the historical control range (0.5-1.5) for all simazine groups.

One 500 mg/kg simazine group, bone marrow at 48 hours, was in the "no decision" range (α and $\beta > 0.10$). The peripheral blood from the same animals was clearly negative. Evaluation of bone marrow smears from four animals dosed with 580 mg/kg of simazine and sacrificed at 48 hours in the preliminary range-finding study yielded negative results. The equivocal result found in one group was not repeated and therefore was not thought to be significant.

In this micronucleus test, sufficient numbers of cells and time points were examined at each dose level, the maximum possible dose was given, and the PCE-RBC ratio was not depressed. The results for all treatment groups were negative. Simazine is therefore negative in the micronucleus test.

Table 295

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

SIMAZINE

Retest

Compound	Metabolic Activation	Compound Added (µg/plate)	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative Control							
DMSO	-					10 13	100 105
	+					32 29	90 116
Positive Control							
2-Anthramine	-	1.0				25	105
	+	1.0				273	488
Sodium Azide	-	0.5					474
2-Nitrofluorene	-	5.0				466	
Simazine	-	500.0				17	100
	-	1,000.0				17	115
	-	2,000.0				14	109
	-	3,000.0				13	102
	-	4,000.0				33	87
	-	5,000.0				13	107
	+	500.0				23	104
	+	1,000.0				25	110
	+	2,000.0				24	120
	+	3,000.0				29	108
	+	4,000.0				24	103
	+	5,000.0				16	100

Table 296

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D7

SIMAZINE

Experiment 1

Compound	MA	% Conc.	Survivors Cells Per ml (× 10⁻⁷)	Survivors %	Crossing-Over: Mitotic Recombinants Per ml (× 10⁻³)	Crossing-Over: Mitotic Recombinants Per 10⁵ Survivors	Crossing-Over: Total Aberrants Per ml (× 10⁻³)	Crossing-Over: Total Aberrants Per 10⁵ Survivors	Gene Conversion: Convertants Per ml (× 10⁻¹)	Gene Conversion: Convertants Per 10⁵ Survivors	Reverse Mutation: Revertants Per ml (× 10⁰)	Reverse Mutation: Revertants Per 10⁵ Survivors
Negative Control												
DMSO	-		6.3	100	0.5	0.8	3.0	4.8	190	30	8.5	1.4
	+		6.3	100	4.0	6.3	6.0	9.5	100	16	8.5	1.4
Positive Controls												
1,2,3,4-Diepoxybutane	-	0.006	2.0	32	35	180	220	1,100	1,700	850	480	240
	+	0.006	3.9	62	36	92	200	510	1,500	380	340	87
Simazine	-	0.05	5.1	81	0	0	0	0	83	16	22	4.3
	-	0.1	6.3	100	2.0	3.2	3.0	4.8	95	15	18	2.9
	-	0.5	5.5	87	1.0	1.8	4.0	7.3	88	16	15	2.7
	-	1.0	6.4	100	3.0	4.7	11	17	110	17	18	2.8
	-	2.5	5.5	87	1.0	1.8	0	7.3	100	18	19	3.5
	+	0.05	5.0	79	2.0	4.0	[illegible]	4.0	76	15	22	4.4
	+	0.1	5.2	83	3.0	5.8	4.0	7.7	87	17	20	3.9
	+	0.5	6.0	95	1.0	1.7	3.0	5.0	98	16	2.0	0.3
	+	1.0	5.7	90	7.0	12	11	19	76	13	19	3.3
	+	2.5	5.3	84	5.0	9.4	9.0	17	87	16	17	3.2

Table 297

IN VITRO ASSAYS WITH *SACCHAROMYCES CEREVISIAE* D7

SIMAZINE

Experiment 2

Compound	MA	% Conc.	Survivors		Crossing-Over				Gene Conversion		Reverse Mutation	
			Cells		Mitotic Recombinants		Total Aberrants		Convertants		Revertants	
			Per ml ($\times 10^{-7}$)	%	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors	Per ml ($\times 10^{-3}$	Per 10^5 Survivors	Per ml ($\times 10^{-1}$)	Per 10^5 Survivors	Per ml ($\times 10^{0}$)	Per 10^5 Survivors
Negative Control												
DMSO	-		6.0	100	0	0	17	28	85	14	42	7.0
	+		5.5	100	1.0	1.8	14	25	81	15	30	5.5
Positive Controls												
1,2,3,4-Diepoxybutane	-	0.006	5.5	92	55	100	1,100	2,000	2,600	470	600	110
	+	0.006	7.0	130	41	59	510	730	1,800	260	270	39
Simazine	-	0.05	5.9	98	0	0	10	17	72	12	28	4.8
	-	0.1	5.5	92	0	0	22	40	56	10	37	6.7
	-	0.5	5.1	85	0	0	12	24	68	13	32	6.3
	-	1.0	5.3	88	0	0	14	26	74	14	26	4.9
	-	2.5	3.0	50	0	0	3.0	10	67	22	22	7.3
	+	0.05	5.0	91	2.0	4.0	19	38	60	12	35	7.0
	+	0.1	5.3	96	0	0	11	21	62	12	41	7.7
	+	0.5	5.7	100	0	0	13	23	64	11	27	4.7
	+	1.0	5.8	110	0	0	11	19	73	13	27	4.7
	+	2.5	2.0	36	0	0	11	55	60	30	26	13

Table 298

DIFFERENTIAL TOXICITY ASSAYS WITH SALMONELLA TYPHIMURIUM STRAINS

Experiment 1

Compound	Amount of Compound Applied to Disc	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		SL4525 (rec^+)		SL4700 (rec^-)		TA1978 ($uvrB^+$)		TA1538 ($uvrB^-$)	
Negative Control									
Kanamycin	100.0 µg	14	13	13	14	13	13	14	14
Positive Controls									
MMS[*]	10.0 mg	47	46	68	65				
4-NQO[†]	2.0 mg					12	12	24	23
Simazine	0.5 mg	6	6	6	6	6	6	6	6
	1.0 mg	6	6	7	7	6	6	8	9

Table 299

DIFFERENTIAL TOXICITY ASSAYS WITH SALMONELLA TYPHIMURIUM STRAINS

Experiment Date: 12 December 1980

Compound	Amount of Compound Applied to Disc	Zone of Inhibition of Growth (in mm, disc = 6 mm) SL4525 (rec$^+$)		SL4700 (rec$^-$)		TA1978 (uvrB$^+$)		TA1538 (uvrB$^-$)	
Negative Control									
Kanamycin	100.0 μg	15	14	16	15	17	17	15	16
Positive Controls									
MMS*	10.0 mg	45	40	72	74				
4-NQO†	2.0 mg					12	12	28	26
Simazine	1.0 mg	7	7	7	7	7	7	7	7
	2.0 mg	7	7	7	7	7	7	7	7

(Continued)

Table 300

CYTOTOXICITY TEST OF CHINESE HAMSTER OVARY CELLS EXPOSED TO SIMAZINE FOR 21.5 HOURS WITHOUT METABOLIC ACTIVATION

Treatment	Number of Divisions in Bromodeoxyuriding (BrdU)*
Negative control (1% DMSO)	M_2
Simazine (mg/ml)	
2.48	M_2
12.39	M_2
61.96	M_2
309.8	M_2
1549†	M_2 >>M_1‡
Positive control (10^{-3} M ethyl methanesulfonate)	M_2

---

* M_1, one dividion in BrdU; M_2, two divisions in BrdU.

† Simazine precipitated in culture medium at these concentrations.

‡ The cell type to the left of the >> sign was greatly in excess.

Table 301

CYTOTOXICITY TEST OF CHINESE HAMSTER OVARY CELLS EXPOSED TO SIMAZINE FOR 2 HOURS WITH METABOLIC ACTIVATION

Treatment	Number of Divisions in Bromodeoxyuridine (BrdU)*
Negative control (1% DMSO)	M_2
Siamzine (mg/ml)	
2.78	M_2
13.9	M_2
69.5	M_2
347.7	M_2
1738.3†	
Positive control (10^{-3} M dimethylnitroszmine)	$M_2 > M_1$‡

---

* M_1, one division in BrdU; M_2, two divisions in BrdU.
† The media foamed when simazine was added at this concentration.
‡ The cell type to the left of the > sign was in excess.

Table 302

SISTER CHROMATID EXCHANGE (SCE) FREQUENCIES IN CHINESE HAMSTER OVARY (CHO) CELLS
EXPOSED TO SIMAZINE FOR 21.5 HOURS WITHOUT METABOLIC ACTIVATION

Treatment	Cytogeneticist[*]	No. of SCEs	No. of Chromosomes	Chromosomes/Cell (Mean ± SE)	SCEs/Cell[†] (Mean ± SE)	SCEs/Chromosome[†] (Mean ± SD)
Negative control (1% DMSO)	A	266	471	18.84±0.94	10.64±0.65	0.565±0.035
	B	270	469	18.76±0.72	10.80±0.66	0.576±0.035
Simazine (mg/ml)						
106.25	A	261	478	19.12±0.78	10.44±0.65	0.546±0.034
	B	252	477	19.08±0.57	10.08±0.63	0.528±0.033
212.5[‡]	A	274	473	18.92±0.57	10.96±0.66	0.579±0.035
	B	264	476	19.04±0.73	10.56±0.65	0.555±0.034
425[‡]	A	286	477	19.08±0.57	11.44±0.68	0.600±0.035
	B	282	482	19.28±0.79	11.28±0.67	0.585±0.035
850[‡]	A	266	479	19.16±0.75	10.64±0.65	0.555±0.034
	B	204	481	19.24±0.57	8.16±0.57	0.424±0.030
1700[‡]	A	315	477	19.08±0.86	12.60±0.71	0.660±0.037
	B	§	--	--	--	--
Positive control (10^{-3} M ethyl methanesulfonate)	A	790	479	19.16±1.25	31.60±1.12	1.649±0.059
	B	902	484	19.36±0.91	36.08±1.20	1.864±0.062

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[*] Each cytogeneticist analyzed 25 cells per sample.

[†] A one-way anaylysis of variance comparing the SCE frequencies in CHO cells exposed to simazine and to the negative control indicated that the variance between treatment groups was not significantly greater than the variance within treatment groups.

[‡] Simazine precipitated in culture medium at these concentrations.

[§] This sample was not analyzed because of poor contrast.

Table 303

SISTER CHROMATID EXCHANGE (SCE) FREQUENCIES IN CHINESE HAMSTER OVARY (CHO) CELLS
EXPOSED TO SIMAZINE FOR 2 HOURS WITH METABOLIC ACTIVATION

Treatment	Scorer	No. of SCEs	No. of Chromosomes	Chromosomes/Cell (Mean ± SE)	SCEs/Cell † (Mean ± SE)	SCEs/ Chromosome † (Mean ± SD)
Negative control	A	284	498	19.12±1.47	11.36±0.67	0.570±0.034
(1% DMSO)	B	258	473	18.92±0.64	10.32±0.64	0.545±0.034
Simazine (mg/ml)						
106.25	A	309	469	18.76±1.33	12.36±0.70	0.659±0.037
	B	297	478	19.12±0.73	11.88±0.69	0.621±0.036
212.5	A	299	472	18.88±1.13	11.96±0.69	0.633±0.037
	B	352	471	18.84±0.75	14.08±0.75	0.747±0.040
425	A	432	490	19.60±1.08	17.28±0.83	0.882±0.042
	B	283	486	19.44±1.00	11.32±0.67	0.582±0.035
850	A	297	487	19.48±1.36	11.88±0.69	0.610±0.035
	B	292	476	19.04±0.68	11.68±0.68	0.613±0.036
1700	A	332	491	19.64±0.91	13.28±0.73	0.676±0.037
	B	277	482	19.28±0.84	11.08±0.67	0.575±0.035
Positive control	A	900	495	19.80±1.12	36.00±1.20	1.818±0.061
(10^{-3} M dimethylnitrosamine)	B	955	479	19.16±0.90	38.20±1.24	1.994±0.065

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* Each cytogeneticist analyzed 25 cells per sample.

† One-way analysis of variance comparing the SCE frequencies in CHO cells exposed to simazine and to the negative control indicated that the variance between treatment groups was not significantly greater than the variance within treatment groups.

Table 304

MOUSE LYMPHOMA FORWARD MUTATION CONCENTRATION-RANGE ASSAY OF SIMAZINE IN THE ABSENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (µg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency ($\times 10^{-6}$)	Induced Mutation Frequency ($\times 10^{-6}$)
Solvent control (1% DMSO)	100.0	600	569	80	100.5	100.5	28	--
	100.0	600	563	110	99.5	99.5	39	--
Positive control (500 µg/ml EMS)	63.9	600	364	968	64.3	41.1	532	498
	74.1	600	366	999	64.7	47.9	546	512
Simazine								
1	105.5	600	603	114	106.5	112.4	38	4
2	95.2	600	582	98	102.8	97.9	34	--
5	100.0	600	507	106	89.6	89.6	42	8
10	106.4	600	528	129	93.3	99.3	49	15
20	95.2	600	582	86	102.8	97.9	30	--
40	82.5	600	528	74	93.3	77.0	28	--
80	95.9	600	498	84	88.0	84.4	34	--
100	84.4	600	537	85	94.9	80.1	32	--
150	PPT*	--	--	--	--	--	--	--
200	PPT	--	--	--	--	--	--	--
300	PPT	--	--	--	--	--	--	--
400	PPT	--	--	--	--	--	--	--
500	PPT	--	--	--	--	--	--	--
600	PPT	--	--	--	--	--	--	--
700	PPT	--	--	--	--	--	--	--
800	PPT	--	--	--	--	--	--	--
900	PPT	--	--	--	--	--	--	--
1000	PPT	--	--	--	--	--	--	--

*Persistent precipitate present after washdown procedure; sample discarded.

Table 305

MOUSE LYMPHOMA FORWARD MUTATION CONCENTRATION-RANGE ASSAY OF SIMAZINE IN THE PRESENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (µg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency ($\times 10^{-6}$)	Induced Mutation Frequency ($\times 10^{-6}$)
Solvent control (1% DMSO)	108.2	600	591	221	99.7	107.8	75	--
	91.8	600	595	202	100.3	92.1	68	--
Positive control (5 µg/ml 3-MCA)	51.8	600	381	570	64.2	33.3	299	227
	51.8	600	387	570	65.3	33.8	295	223
Simazine								
1	91.8	600	550	255	92.7	85.1	93	21
2	95.1	600	518	222	87.4	83.1	86	14
5	98.4	600	586	259	98.8	97.2	88	16
10	90.2	600	606	279	102.2	92.2	92	20
20	84.3	600	590	245	99.5	83.9	83	11
40	84.3	600	633	272	106.7	90.0	86	14
80	72.4	600	571	291	96.3	69.7	102	30
100	65.5	600	507	317	85.5	56.0	125	53
150	52.5	600	523	371	88.2	46.3	142	70
200	51.2	600	598	344	100.8	51.6	115	43
300	37.7	600	580	325	97.8	36.9	112	40
400	PPT*	--	--	--	--	--	--	--
500	PPT	--	--	--	--	--	--	--
600	PPT	--	--	--	--	--	--	--
700	PPT	--	--	--	--	--	--	--
800	PPT	--	--	--	--	--	--	--
900	PPT	--	--	--	--	--	--	--
1000	PPT	--	--	--	--	--	--	--

* Persistent precipitate present after washdown procedure; sample discarded

Table 306

MOUSE LYMPHOMA FORWARD MUTATION ASSAY OF SIMAZINE IN THE ABSENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (µg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency (× 10^{-6})	Induced Mutation Frequency (× 10^{-6})
Solvent control (1% DMSO)	99.9	600	495	81	96.5	96.4	33	--
	100.1	600	531	88	103.5	103.6	33	--
Positive control (500 µg/ml EMS)	63.5	600	302	889	58.9	37.4	589	556
	59.5	600	289	858	56.3	33.5	594	561
Simazine								
20	95.8	NC*	--	--	--	--	--	--
	88.7	NC	--	--	--	--	--	--
100	85.4	NC	--	--	--	--	--	--
	95.7	NC	--	--	--	--	--	--
128	92.1	NC	--	--	--	--	--	--
	85.4	NC	--	--	--	--	--	--
152	85.4	NC	--	--	--	--	--	--
	92.1	NC	--	--	--	--	--	--
200	85.4	NC	--	--	--	--	--	--
	97.2	NC	--	--	--	--	--	--
228	92.1	600	592	142	115.4	106.3	48	15
	89.3	600	572	120	111.5	99.6	42	9
252	87.5	600	629	134	122.6	107.3	43	10
	89.3	600	607	124	118.3	105.7	41	8
300	89.3	600	614	94	119.7	106.9	31	--
	97.2	600	537	131	104.7	101.7	49	16
356	89.3	600	662	121	129.0	115.2	37	4
	93.5	600	550	146	107.2	100.2	53	20
400	102.1	600	523	202	101.9	104.1	77	44
	91.9	600	557	114	108.6	99.8	41	8

*Not cloned

Table 307

MOUSE LYMPHOMA FORWARD MUTATION ASSAY OF SIMAZINE IN THE PRESENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency ($\times 10^{-6}$)	Induced Mutation Frequency ($\times 10^{-6}$)
Solvent control (1% DMSO)	97.9	600	574	194	95.0	93.0	68	--
	102.1	600	634	255	105.0	107.2	80	--
Positive control (4 mg/ml 3-MCA)	89.0	600	554	720	91.7	81.6	260	186
	83.8	600	--*	--*	--	--	--	--
Simazine								
5	97.1	600	584	182	96.7	93.9	62	--
	91.0	600	573	178	94.9	86.3	62	--
20	93.2	600	616	228	102.0	95.1	74	--
	93.2	600	600	255	99.3	92.6	85	11
50	85.6	600	581	262	96.2	82.3	90	16
	87.2	600	562	253	93.0	81.1	90	16
80	80.3	600	667	274	110.4	88.7	82	8
	89.3	600	627	294	103.8	92.7	94	20
100	77.3	600	650	324	107.6	83.2	100	26
	83.7	600	589	332	97.5	81.6	113	39
150	79.2	600	581	367	96.2	76.2	126	52
	82.0	600	546	326	90.4	74.1	119	45
300	74.4	600	577	429	95.5	71.1	149	75
	70.6	600	587	443	97.2	68.6	151	77
350	71.7	600	--*	432	--	--	--	--
	68.0	600	--*	387	--	--	--	--
400	PPT†	--	--	--	--	--	--	--
	PPT	--	--	--	--	--	--	--
500	PPT	--	--	--	--	--	--	--
	PPT	--	--	--	--	--	--	--

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*Sample lost

†Persistent precipitate present after washdown procedure; sample discarded

Table 308

DOSAGE AND SACRIFICE SCHEDULE

Compound	No. Animals	Dose at 0 and 24 Hr	Mean Body Weight Change (g ± SD)	No. Sacrificed			No. Fatalities
				30 Hr	48 Hr	72 Hr	
TMP--Positive control	16	1.25 g/kg	-2.3 ± 0.9	4	4	4	2
Water--Negative control	36	10 ml/kg	-0.3 ± 1.1	10	10	10	0
Simazine							
Low Dose	36	125 mg/kg	-0.01 ± 0.9	8	8	8	3 (2+)
Medium Dose	36	250 mg/kg	-0.4 ± 0.9	8	8	8	1 (1+)
High Dose	36	500 mg/kg	-1.3 ± 1.6	8	8	8	0 (1+)

+The number in parentheses indicates animals that died due to obvious misdosing.

Table 309

INCIDENCE OF MICRONUCLEATED POLYCHROMATOPHILIC ERYTHROCYTES IN BLOOD AND BONE MARROW
(500 PCEs Scored per Smear)

Dose	30 Hours Animal No.	30 Hours Blood PCE with MN	30 Hours BM PCE with MN	30 Hours BM PCE/RBC	48 Hours Animal No.	48 Hours Blood PCE with MN	48 Hours BM PCE with MN	48 Hours BM PCE/RBC	72 Hours Animal No.	72 Hours Blood PCE with MN	72 Hours BM PCE with MN	72 Hours BM PCE/RBC
Water 10 ml/kg	10	1	0	1.3	20	2	0	1.3	2R	0	2	1.0
	1R	2	1	1.4	40	0	2	0.8	2L	0	1	1.4
	1L	2	0	1.5	4R	3	2	0.8	2B	0	1	1.1
	1B	0	0	1.7	4L	0	0	1.0	70	1	1	0.9
	3R	1	1	0.9	4B	1	1	0.9	7R	0	1	0.7
	3L	1	1	0.8	5B	2	1	0.5	7L	0	0	0.7
	3B	1	0	1.2	60	0	2	1.1	80	2	0	0.7
	50	2	0	3.1	6R	1	0	1.4	8R	1	2	1.2
	5R	1	2	2.7	6L	0	0	0.4	8L	1	0	1.0
	5L	ns	2	1.0	6B	2	0	0.7	8B	2	2	1.0
	Total	11	7			11	8			7	10	
	Mean			1.6±.8				0.9±.3				1.0±.2
Trimethyl Phosphate 1.25 g/kg	120	12	20	0.4	100	10	22	0.2	110	16	10	0.3
	12R	10	31	0.4	10R	8	14	0.3	11L	10	6	0.4
	12L	13	28	0.4	10L	27	23	0.5	13L	0	4	1.1
	12B	0	31	0.3	10B	13	20	0.3	13B	3	5	0.6
	Total	35	110			58	79			29	25	
	Mean			0.4±.05				0.3±.1				0.6±.4
Simazine 125 mg/kg	470	ns	1	0.6	490	0	0	1.0	49L	1	2	0.7
	47R	ns	1	0.7	510	0	0	0.9	49B	1	0	0.9
	47L	ns	0	1.1	51R	3	1	0.8	500	1	0	1.0
	480	0	2	0.8	51L	0	2	1.3	50R	0	0	0.9
	48R	2	1	0.8	51B	1	1	1.0	530	0	1	0.7
	48L	1	4	1.7	520	2	0	0.9	53R	0	0	0.9
	48B	1	1	2.7	52R	1	1	1.2	53L	1	1	0.8
	55R	ns	0	1.0	52B	2	1	0.7	53B	0	0	0.4
	Total	4	10			9	6			4	4	
	Mean			1.2±.7				1.0±.2				0.8±.2
Simazine 250 mg/kg	580	1	5	0.9	590	0	1	1.1	560	0	0	0.7
	58B	3	2	2.0	59R	0	0	0.8	56R	0	0	0.5
	60R	0	0	1.0	59L	0	0	0.7	56L	0	1	0.7
	60L	1	1	1.1	59B	1	2	0.7	570	0	0	1.1
	60B	0	1	1.6	640	0	0	0.9	57R	0	0	1.1
	61R	3	1	1.0	64R	0	1	0.9	57L	1	0	0.7
	61L	0	1	0.8	64L	0	1	0.9	57B	0	0	1.4
	61B	1	1	1.3	64B	0	0	1.5	58L	1	2	0.6
	Total	9	12			1	5			2	3	
	Mean			1.2±.4				0.9±.3				0.85±.3
Simazine 500 mg/kg	690	1	0	1.6	660	1	3	0.7	650	0	1	0.7
	69R	1	0	1.2	66R	1	4	0.9	65R	0	1	1.8
	69L	1	0	0.7	66L	0	3	0.3	65L	1	0	1.1
	69B	0	1	1.2	66B	1	0	0.9	65B	1	0	1.3
	700	0	0	0.6	670	0	2	1.1	720	0	0	0.3
	70R	1	0	0.6	67R	3	1	1.6	730	0	0	0.8
	70L	0	3	0.8	67L	2	1	2.1	73R	0	1	1.0
	70B	1	1	1.5	67B	0	1	1.9	73B	0	2	0.7
	Total	5	5			8	15			2	5	
	Mean			1.0±.4				1.2±.6				1.0±.5

PCE = polychromatophilic erythrocyte; RBC = mature erythrocyte; MN = micronucleus; BM = bone marrow; ns = no slide evaluated.

2,4,5-T

S. typhimurium Plate Incorporation, E. coli WP-2 (Tables 310, 311). DMSO was used as the solvent in all the experimental procedures. 2,4,5-T Acid was tested twice in duplicate, on separate days, over a dose range of 25 to 5000 µg/plate and was found to be negative. Toxicity was observed at > 2500 µg/plate in the absence of metabolic activation with all strains. 2,4,5-T Acid was less toxic when the metabolic activation system was included.

S. cerevisiae D3 (Tables 312-315). 2,4,5-T Acid was tested on four separate days at concentrations ranging from 0.05 to 0.75% and once over a range of 0.2 to 1.0%. In the first experiment (Table 312), the compound appeared to cause a dose-related increase in the number of mitotic recombinants per 10^5 survivors with and without metabolic activation. However, in subsequent experiments, dose-related increases in recombinants per 10^5 survivors were not observed although sporadic elevations were noted. Thus, we conclude that 2,4,5-T was negative with and without activation.

E. coli/B. subtilis Relative Toxicity (Table 316). Sonification was used to dissolve the compound in DMSO. 2,4,5-T Acid was found to be reproducibly more toxic to the rec^- B. subtilis strain than to the rec^+ strain, but was equally toxic to the $polA^-$ and $polA^+$ E. coli strains. 2,4,5-T Acid apparently causes DNA damage that is repaired by the rec function but is not dependent on the polA function for repair. Thus, this compound was negative in the E. coli system, but positive in the B. subtilis system.

Table 310

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

2,4,5-T Acid

Experiment 1

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate TA1535		TA1537		TA1538		TA98		TA100		Tryptophan Revertants per Plate WP2	
Negative control (DMSO)	-		14	21	10	7	10	7	17	18	89	86	31	36
	+		5	11	10	12	17	26	28	29	87	84	48	37
Positive controls														
Sodium azide	-	1.0	474	430							557	549		
9-Aminoacridine	-	50.0			207	272								
2-Nitrofluorene	-	5.0					1118	1024	532	638				
AF2	-	0.1											990	1130
2-Anthramine	-	1.0					15	14	20	18	102	95		
	+	1.0					256	252	257	284	475	501		
	-	2.5	19	22	7	6								
	+	2.5	256	13	63	75								
	-	10.0											33	38
	+	10.0											989	1154
2,4,5-T Acid	-	25	24	30	6	12	17	12	17	18	75	101	33	43
	-	50	20	17	5	7	10	14	24	23	89	92	30	43
	-	100	27	26	5	10	17	8	26	20	87	87	36	51
	-	500	20	24	5	7	13	11	19	22	74	85	50	45
	-	1000	18	25	8	6	12	9	21	19	78	59	30	51
	-	2500	Toxic		Toxic		Toxic		Toxic		Toxic		Toxic	
	-	5000	Toxic		Toxic		Toxic		Toxic		Toxic		Toxic	
	+	25	12	11	26	19	11	23	34	34	104	90	51	41
	+	50	8	20	21	20	24	22	39	42	86	73	61	50
	+	100	18	8	14	21	24	30	28	36	88	87	40	42
	+	500	11	11	18	19	21	20	38	31	85	111	42	52
	+	1000	12	13	15	12	19	18	36	34	81	87	49	48
	+	2500	8	10	7	9	21	13	24	23	C*	67	29	26
	+	5000	8	6	Toxic		Toxic		23	24	Toxic		Toxic	

---

*C, contaminated.

Table 311

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

2,4,5-T Acid

Experiment 2

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate										Tryptophan Revertants per Plate	
			TA1535		TA1537		TA1538		TA98		TA100		WP2	
Negative control (DMSO)	-		37	37	17	6	15	7	29	33	127	122	29	44
	+		8	19	8	14	19	26	42	49	126	111	54	50
Positive controls														
Sodium azide	-	0.5	356											
	-	1.0									537			
9-Aminoacridine	-	50.0			506									
2-Nitrofluorene	-	5.0					872		489					
AF2	-	0.1											1927	1988
2-Anthramine	-	1.0					14		30		120			
	+	1.0					238		262		239			
	-	2.5	28		9									
	+	2.5	86		49									
	-	10.0											44	55
	+	10.0											480	474
2,4,5-T Acid	-	25	30	36	10	7	14	12	24	20	139	100	33	29
	-	50	33	27	10	9	16	13	15	17	125	122	27	36
	-	100	43	32	11	10	14	15	24	29	136	115	45	37
	-	500	32	44	7	5	17	11	20	17	125	126	29	42
	-	1000	25	23	10	11	10	14	19	13	103	103	32	30
	-	2500	Toxic		Toxic		Toxic		Toxic		Toxic		29	C*
	-	5000	Toxic		Toxic		Toxic		Toxic		Toxic		27	Toxic
	+	25	18	14	21	17	18	29	30	34	112	115	31	45
	+	50	13	14	18	23	26	34	40	35	97	130	52	39
	+	100	16	15	20	18	30	19	33	33	126	117	45	37
	+	500	14	19	22	16	24	25	33	36	100	98	45	37
	+	1000	7	14	14	11	28	29	37	30	101	109	42	30
	+	2500	13	16	Toxic		12	13	29	32	85	56	30	41
	+	5000	6	7	Toxic		Toxic		Toxic		Toxic		Toxic	

---

*C, contaminated.

Table 312

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

2,4,5-T Acid

Experiment 1

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml (x 10^{-7})	Percent	Per ml (x 10^{-3})	Per 10^5 Survivors
Negative control (DMSO)	-		4.7	100	3.5	7.5
	+		6.1	100	1.5	2.5
Positive control 1,2,3,4-Diepoxybutane	-	0.025	4.9	104	913	1900
	+	0.025	4.2	69	723	1700
2,4,5-T Acid	-	0.05	4.3	91	7.0	16
	-	0.1	5.4	115	4.0	7.4
	-	0.25	5.8	123	11.0	19
	-	0.5	5.8	123	18.0	31
	-	0.75	1.2	26	24.0	200
	+	0.05	6.5	107	7.0	11
	+	0.1	5.7	93	4.0	7.0
	+	0.25	5.8	95	4.0	6.9
	+	0.5	3.8	62	16.0	42
	+	0.75	0.6	10	10.0	170

Table 313

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

2,4,5-T Acid

Experiment 2

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control (DMSO)	-		6.3	100	3.5	5.6
	+		7.9	100	2.0	2.5
Positive control	-	0.025	6.1	97	708	1200
1,2,3,4-Diepoxybutane	+	0.025	6.3	80	883	1400
2,4,5-T Acid	-	0.05	7.0	111	7.0	10
	-	0.1	5.1	81	4.0	7.8
	-	0.25	8.7	138	2.0	2.3
	-	0.5	6.0	95	5.0	8.3
	-	0.75	4.8	76	7.0	14
	+	0.05	7.4	94	5.0	6.8
	+	0.1	8.5	108	5.0	5.9
	+	0.25	6.7	85	5.0	7.5
	+	0.5	9.0	114	6.0	6.7
	+	0.75	4.9	62	3.0	6.1

Table 314

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

2,4,5-T Acid

Experiment 3

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml (x 10^{-7})	Percent	Per ml (x 10^{-3})	Per 10^5 Survivors
Negative control (DMSO)	-		5.8	100	7.0	12
	+		5.9	100	4.0	6.8
Positive control 1,2,3,4-Diepoxybutane	-	0.025	7.4	128	620	840
	+	0.025	3.5	59	343	980
2,4,5-T Acid	-	0.05	6.1	105	7.0	12
	-	0.1	5.6	97	2.0	3.6
	-	0.25	6.4	110	4.0	6.3
	-	0.5	6.3	109	4.0	6.4
	-	0.75	4.3	74	2.0	4.7
	+	0.05	6.4	108	6.0	9.4
	+	0.1	5.4	92	1.0	1.9
	+	0.25	4.8	81	3.0	6.3
	+	0.5	5.2	88	5.0	9.6
	+	0.75	4.5	76	14.0	31

Table 315

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

2,4,5-T Acid

Experiment 4

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml (x 10^{-7})	Percent	Per ml (x 10^{-3})	Per 10^5 Survivors
Negative control	-		8.6	100	5.5	6.4
	+		9.4	100	4.5	4.8
Positive control	-	0.025	5.0	58	580	1200
1,2,3,4-Diepoxybutane	+	0.025	6.5	69	928	1400
2,4,5-T Acid	-	0.2	8.9	103	4.0	4.5
	-	0.4	9.3	108	5.0	5.4
	-	0.6	6.8	79	6.5	9.6
	-	0.7	5.5	64	4.0	7.3
	-	0.8	5.4	63	5.0	9.3
	-	1.0	2.5	29	3.0	12
	+	0.2	8.0	85	6.5	8.1
	+	0.4	10.0	106	4.0	4.0
	+	0.6	9.9	105	5.0	5.1
	+	0.7	5.3	56	4.5	8.5
	+	0.8	5.2	55	3.0	5.8
	+	1.0	1.9	20	5.0	26

Table 316

DIFFERENTIAL TOXICITY ASSAYS
2.4.5-T ACID

Compound	Amount	Zone of Inhibition of Growth (in mm, disc=6 mm)							
		E. coli				B. subtilis			
		W3110 (polA$^+$)		p3478 (polA$^-$)		H17 (rec$^+$)		M45 (rec$^-$)	
EXPERIMENT 1									
Negative control Ampicillin	0.01 mg	20	20	18	17	25	26	28	27
Positive control Methylmethane sulfonate	1.0 mg	30	30	35	35	14	14	46	49
2,4,5-T Acid	1.0	7	7	7	8	17	16	20	20
	5.0	8	8	8	3	14	11	16	18
EXPERIMENT 2									
Negative control Ampicillin	0.01 mg	19	18	17	17	25	25	27	27
Positive control Methylmethane sulfonate	1.0 mg	16	16	22	23	15	15	50	50
2,4,5-T Acid	1.0	6	6	6	7	14	14	19	19
	5.0	9	9	9	9	14	14	19	19

(Continued)

Triallate

S. typhimurium Plate Incorporation (Tables 317, 318). Triallate was previously tested and found positive only with activation (54). Under the present contract it was first tested over a dose range of 0.005 to 1.0 µℓ/plate and later retested from 0.01 to 1.0 µℓ/plate. A mutagenic response, which was reproducible and dose-related, was obtained with strains TA1535, TA98, and TA100 with metabolic activation. Triallate appeared to cause more reversion with strains TA1535 and TA100 than with TA98. Toxicity, denoted by the appearance of pinpoint colonies on the background, was observed in Experiment 2 at 1.0 µℓ/plate with strains TA1537, TA1538 and TA100 in the absence of metabolic activation.

E. coli WP-2. Diallate was previously tested and found to be negative with and without activation in this system (54).

S. cerevisiae D3. This compound was previously tested and found to be positive only with activation in this test system (54).

S. cerevisiae D7 (Tables 319-321). Triallate did not induce mitotic crossing-over, mitotic gene conversion or reverse mutation. Triallate was tested from 0.00075 to 0.0075% in Experiment 1, from 0.006 to 0.01 in Experiment 2, and from 0.00075 to 0.004% in Experiment 3. The increase in the frequency of crossing-over and gene conversion was due to the reduction in number of survivors and does not appear to indicate genetic damage.

L5178Y Mouse Lymphoma (Tables 322-326). Table 322 presents the results of the concentration-range tests of triallate in the absence of metabolic activation. The results showed that the range to use in the mutagenesis assay was approximately 10.0 µg/ml to 60.0 µg/ml. This determination was based on relative total growth values of 98.1% at 10.0 µg/ml and 18.6% at 60.0 µg/ml. Concentration-range testing in the presence of metabolic activation (Table 323) showed that the range to use in mutagenesis testing was approximately 8.0 µg/ml to 20.0 µg/ml, based on the relative total growth values of 96.7% at 8 µg/ml and 15.9% at 20.0 µg/ml.

Tables 324 and 325 present the results of mutagenesis testing of triallate in the absence of metabolic activation. In the first assay, this compound was extremely toxic at concentrations greater than 60.0 µg/ml, yielding relative total growth values of less than 8.9%. In the second assay, the highest concentration of 60.0 µg/ml yielded an average relative total growth value of 13.0%. No significant depression in the relative total growth value was observed at or below 12.0 µg/ml in the first assay and 27.0 µg/ml in the second assay; these concentrations yielded relative total growth values in excess of 80.0%. From approximately 12.0 to 60.0 µg/ml, evidence of a concentration-related increase in induced mutation frequency was observed in both assays. The first assay had one sample that clearly showed a mutation frequency at least twice the spontaneous frequency. In the second assay, three samples in the two highest concentrations showed mutation frequencies at least twice the spontaneous frequency, with relative total growth values of over 10.0%. These results indicate a positive mutagenic response.

Table 326 presents the results of mutagenesis testing of triallate in the presence of metabolic activation. The highest concentration of 24.0 µg/ml yielded an average relative total growth value of 12.4%. No significant depression in the relative total growth was observed at or below 9.0 µg/ml, which yielded an average relative total growth value of 81.2%. From 12.4 to 24.0 µg/ml, a concentration-related increase in induced mutation frequency was observed. A mutation frequency of at least twice the spontaneous frequency was observed at the two highest concentrations, having relative total growth values of over 10.0%. These results indicate a positive mutagenic response.

These combined results provide evidence that triallate increased the mutation frequencies, in a concentration-related manner, above the spontaneous control frequencies. The presence of the metabolic activation system was not required to elicit the positive mutagenic response, but it did appear to enhance the cytotoxicity and mutagenicity of triallate.

Drosophila SLRL (Tables 327-329). Table 325 presents the effects of triallate on the viability and fertility of y B/y$^+$ males. The experimental design of the wide-range pretest called for eight 1:5 dilutions of triallate. Dilution errors caused a slight deviation from this plan. The actual mean daily concentrations of triallate to which the flies were exposed for 3 days are given in Table 327. Green guts were observed in males exposed to each test concentration of triallate, indicating that palatability was not a problem. The wide-range pretest demonstrated that flies were killed by exposure to triallate at concentrations of $\geq$ 0.003% for 3 days. A concentration between 0.003% and 0.0007% triallate appeared to represent an LD_{50}. Fertility in surviving males through production of third instar larvae was good.

The narrow-range pretest was performed to select the most appropriate dose more precisely. In this experiment, a concentration of 0.004% triallate killed all the flies. At a concentration of 0.002% triallate, 60% survived, and all of these males were fertile. At a concentration of 0.001% triallate, all survived and all were fertile. Therefore, 0.002% triallate was chosen as the concentration expected to approximate an LD_{50}.

Table 328 summarizes the results of the testing of triallate for SLRL mutations. Triallate was tested three times with concurrent positive and negative controls. When 0.002% triallate proved excessively toxic in Experiment 1, 0.001% triallate was tested in Experiments 2 and 3 to achieve a sample size that was adequate to detect an 0.2% increase in the mutation frequency. In Experiments 2 and 3, a total of 8341 X-chromosomes from the gametes of 209 triallate-fed Drosophila melanogaster males were sampled for mutagenicity. In the concurrent solvent control group, 7025 chromosomes from 138 male flies were tested; in the positive control (0.01% EMS) group, 280 chromosomes from the 19 treated males were assessed.

Table 329 presents the SLRL mutation frequencies observed in y B/y$^+$Y males fed 0.002% or 0.001% triallate for 3 days and in concurrently tested positive and solvent controls. Clustered lethals consisting of more than three mutations derived from one treated male in the triallate or solvent control groups were not included in the computation because of their probable origins as spermatogonial events. The overall SLRL mutation frequencies obtained for Experiments 2 and 3 were 0.28% for the solvent control group, 0.31% for triallate, and 22.9% for the positive control group. The difference in mutation frequencies between the triallate and solvent control groups was

0.03%. When these results were analyzed using the Kastenbaum-Bowman test, the increased mutation frequency observed in the triallate-fed Drosophila was not significant.

This study was not designed to sample separate broods (representing chemical exposure at various stages during spermatogenesis) in sufficient numbers to establish the statistical significance of any observed differences. Rather, a brooding scheme was selected to provide preliminary evidence for or against a marked stage-specific sensitivity to a test chemical. Positive results might suggest the need for further study.

Broods A, B, and C represent, respectively, gametes that were primarily mature sperm, spermatids, and spermatocytes undergoing meiosis at the termination of exposure to the chemical (47). Among the triallate-fed flies, slightly higher mutation frequencies were observed in Broods A and B than in Brood C. Only in Brood A were the mutation frequencies observed in triallate-fed flies consistently greater than solvent control mutation frequencies.

In conclusion, the herbicide triallate fed to Drosophila males at a concentration of 0.001 to 0.002% for 3 days caused no significant increase in the SLRL mutation frequency compared with the solvent control group.

Table 317

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

TRIALLATE

Experiment 1

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate									
			TA1535		TA1537		TA1538		TA98		TA100	
Negative Control												
DMSO	-	50.0 µl	29	21	20	18	15	12	32	38	116	108
	+	50.0	17	17	24	20	9	15	31	32	112	115
Positive Controls												
Sodium Azide	-	0.5 µg	233	247								
	-	1.0									347	349
9-Aminoacridine	-	50.0			135	150						
2-Nitrofluorene	-	5.0					768	821	446	427		
2-Anthramine	-	1.0					18	17	20	26	131	142
	+	1.0					187	238	245	260	592	493
	-	2.5	26	26	33	18						
	+	2.5	195	207	149	125						
Triallate	-	0.005 µl	31	20	12	17	13	13	41	39	110	125
	-	0.01	24	25	15	9	17	16	30	37	112	104
	-	0.05	31	20	16	17	12	15	26	40	125	103
	-	0.1	26	28	17	14	18	13	45	44	115	103
	-	0.5	30	51	8	6	10	13	32	40	127	114
	-	1.0	33	40	8	13	14	14	21	19	135	116
	+	0.005 µl	7	21	36	28	36	37	49	40	124	136
	+	0.01	19	18	30	41	37	16	42	40	135	140
	+	0.05	39	50	21	37	24	29	44	60	276	269
	+	0.1	76	76	25	30	28	29	56	75	457	489
	+	0.5	313	324	16	21	28	33	122	116	1136	1406
	+	1.0	459	492	32	21	34	36	163	185	1728	1851

Table 318

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

TRIALLATE

Experiment 2

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate									
			TA1535		TA1537		TA1538		TA98		TA100	
Negative Control												
DMSO	-	50.0 µl	29	27	16	12	21	17	33	26	128	155
	+	50.0	38	30	31	29	30	30	39	56	158	140
Positive Controls												
Sodium Azide	-	0.5 µg	375	398								
	-	1.0									564	537
9-Aminoacridine	-	50.0			373	415						
2-Nitrofluorene	-	5.0					824	908	429	420		
2-Anthramine	-	1.0					18	26	33	40	143	126
	+	1.0					402	514	341	400	709	759
	-	2.5	30	33	20	18						
	+	2.5	259	253	128	128						
Triallate												
	-	0.01 µl	44	25	25	26	15	16	32	29	129	136
	-	0.05	38	36	24	6	18	15	40	31	116	124
	-	0.1	32	24	6	14	7	18	28	29	100	129
	-	0.25	30	33	8	20	18	12	15	30	102	147
	-	0.5	29	26	6	15	8	17	19	27	135	116
	-	1.0	32	40	pp*	pp	pp	pp	24	39	pp	pp
	+	0.01 µl	37	44	30	21	34	25	48	57	175	166
	+	0.05	54	54	38	25	32	19	44	53	311	299
	+	0.1	99	98	25	26	29	25	48	68	625	617
	+	0.25	198	212	33	21	33	20	97	89	1105	1079
	+	0.5	337	369	28	18	27	31	130	134	1501	1588
	+	1.0	526	538	15	17	29	31	179	171	1792	1650

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*pp=pinpoint colonies

Table 319

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D7

TRIALLATE

Experiment 1

Compound	MA	% Conc.	Survivors		Crossing-Over				Gene Conversion		Reverse Mutation	
					Mitotic Recombinants		Total Aberrants		Convertants		Revertants	
			Cells Per ml ($\times 10^{-7}$)	%	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors	Per ml ($\times 10^{-1}$)	Per 10^6 Survivors	Per ml ($\times 10^{0}$)	Per 10^7 Survivors
Negative Control												
DMSO	-	10.0	5.2	100	4.5	8.7	6.5	13	70	13	14	2.7
	+	10.0	5.6	100	1.5	2.7	11	20	66	12	16	2.9
Positive Control												
1,2,3,4-Diepoxybutane	-	0.013	3.9	75	96	250	250	640	1900	490	280	72
	+	0.013	3.7	66	92	250	370	1000	1600	430	330	89
Triallate	-	0.00075	5.7	110	0	0	9.0	16	58	10	13	2.3
	-	0.001	5.2	100	0	0	19	37	44	8.5	15	2.9
	-	0.0025	5.5	106	6.0	11	7.0	13	63	11	13	2.4
	-	0.005	6.1	117	2.0	3.3	12	20	73	12	9.0	1.5
	-	0.0075	3.6	69	1.0	2.8	7.0	19	54	15	2.0	0.56
	+	0.00075	4.9	88	0	0	19	39	48	9.8	11	2.2
	+	0.001	6.0	107	2.5	4.2	31	52	71	12	20	3.3
	+	0.0025	5.5	98	6.0	11	9.0	16	57	10	18	3.3
	+	0.005	5.2	93	2.0	3.8	16	31	64	12	22	4.2
	+	0.0075	5.3	95	1.0	1.9	27	51	65	12	20	3.8

Table 320

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D7

TRIALLATE

Experiment 2

			Survivors		Crossing-Over				Gene Conversion		Reverse Mutation	
					Mitotic Recombinants		Total Aberrants		Convertants		Revertants	
Compound	MA	% Conc.	Cells Per ml ($\times 10^{-7}$)	%	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors	Per ml ($\times 10^{-1}$)	Per 10^6 Survivors	Per ml ($\times 10^0$)	Per 10^7 Survivors
Negative Control												
DMSO	-	10.0	3.5	100	1.0	2.9	8.0	23	61	17	20	5.7
	+	10.0	3.5	100	1.0	2.9	11	31	54	15	22	6.3
Positive Control												
1,2,3,4-Diepoxybutane	-	0.013	1.5	43	67	450	340	2300	1000	670	450	300
	+	0.013	2.3	66	44	190	370	1600	1200	520	410	180
Triallate	-	0.006	2.9	83	0	0	26	90	50	17	16	5.5
	-	0.007	0.95	27	1.0	11	12	130	18	19	6.0	6.3
	-	0.008	0.33	9.4	2.0	61	10	300	8.0	24	1.0	3.0
	-	0.009	T		T		T		T		T	
	-	0.01	T		T		T		T		T	
	+	0.006	3.7	106	0	0	9.0	24	57	15	30	8.1
	+	0.007	4.0	114	3.0	7.5	15	38	60	15	32	8.0
	+	0.008	3.8	109	0	0	18	47	72	19	38	10
	+	0.009	2.2	63	1.0	4.5	18	82	61	28	33	15
	+	0.01	3.1	89	1.0	3.2	27	87	75	24	29	9.4

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*T = toxic

Table 321

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D7

TRIALLATE

Experiment 3

Compound	MA	% Conc.	Survivors		Crossing-Over				Gene Conversion		Reverse Mutation	
					Mitotic Recombinants		Total Aberrants		Convertants		Revertants	
			Cells Per ml ($\times 10^{-7}$)	%	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors	Per ml ($\times 10^{-1}$)	Per 10^6 Survivors	Per ml ($\times 10^0$)	Per 10^7 Survivors
Negative Control												
DMSO	-	10.0	7.7	100	1.0	1.3	13	17	80	10	18	2.3
	+	10.0	8.4	100	0	0	13	15	72	8.6	28	3.3
Positive Control												
1,2,3,4-Diepoxybutane	-	0.013	3.8	49	120	320	580	1500	1400	370	83	22
	+	0.013	6.3	75	93	150	600	950	2300	370	160	25
Triallate	-	0.00075	4.9	64	2.0	4.1	18	37	62	13	14	2.9
	-	0.001	5.4	70	3.0	5.6	13	24	75	14	10	1.9
	-	0.002	5.3	69	1.0	1.9	13	25	49	9.2	10	1.9
	-	0.003	5.2	68	2.0	3.8	23	44	45	8.7	7.0	1.3
	-	0.004	5.1	66	4.0	7.8	26	51	46	9.0	2.0	0.39
	+	0.00075	5.5	65	3.0	5.5	31	56	52	9.5	16	2.9
	+	0.001	5.7	68	3.0	5.3	23	40	62	11	5.0	0.88
	+	0.002	5.9	70	2.0	3.4	20	34	28	4.7	13	2.2
	+	0.003	5.3	63	1.0	1.9	16	30	51	9.6	12	2.3
	+	0.004	5.3	63	3.0	5.7	25	47	67	13	2.0	0.38

Table 322

MOUSE LYMPHOMA FORWARD MUTATION CONCENTRATION-RANGE ASSAY OF TRIALLATE IN THE ABSENCE OF METABOLIC ACTIVATION

Concentration (µg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Calculated Mutation Frequency (X 10^{-6})	Induced Mutation Frequency (X 10^{-6})
Solvent control	102.2	600	561	60	99.0	101.2	21	--
(1% OMSO)	97.8	600	572	111	101.0	98.7	39	--
Positive control	66.3	600	346	823	61.1	40.5	476	446
(500 µg/ml EMS)	69.4	600	327	796	57.7	40.1	487	457
Triallate								
0.5	113.3	600	535	104	94.4	107.0	39	9
1	107.9	600	484	71	85.4	92.2	29	--
2	107.2	600	577	114	101.9	109.2	40	10
5	97.2	600	402	107	71.0	69.0	53	23
8	92.7	600	574	88	101.3	93.9	31	1
10	107.3	600	518	132	91.4	98.1	51	21
20	88.5	600	552	107	97.4	86.2	39	9
30	82.2	600	445	85	78.6	64.6	38	8
50	37.1	600	534	102	94.3	35.0	38	8
60	22.5	600	468	134	82.6	18.6	57	27
80	2.7	NC*	--	--	--	--	--	--
100	0.4	NC	--	--	--	--	--	--
150	0.1	NC	--	--	--	--	--	--
200	0.1	NC	--	--	--	--	--	--
250	0.0	NC	--	--	--	--	--	--
300	0.1	NC	--	--	--	--	--	--
400	0.2	NC	--	--	--	--	--	--
500	0.3	NC	--	--	--	--	--	--

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*Not cloned.

Table 373

MOUSE LYMPHOMA FORWARD MUTATION CONCENTRATION-RANGE ASSAY OF TRIALLATE IN THE PRESENCE OF INDUCED METABOLIC ACTIVATION

Concentration (µg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Calculated Mutation Frequency (X 10^{-6})	Induced Mutation Frequency (X 10^{-6})
Solvent control (1% DMSO)	100.0	600	445	216	95.8	95.8	97	--
	100.0	600	484	240	104.2	104.2	99	--
Positive control (4.0 µg/ml 3-MCA)	69.2	600	367	547	79.0	54.7	298	200
	65.0	600	328	559	70.6	45.9	341	243
Triallate								
0.5	105.9	600	543	251	116.9	123.8	92	--
1	102.3	600	627	319	135.0	138.1	102	4
2	92.8	600	566	305	121.9	113.1	108	10
5	98.8	600	508	297	109.4	108.1	117	19
8	87.9	600	511	317	110.0	96.7	124	26
10	62.1	600	457	325	98.4	61.1	142	44
20	23.3	600	317	394	68.2	15.9	249	151
30	4.3	NC*	--	--	--	--	--	--
50	0.7	NC	--	--	--	--	--	--
60	0.4	NC	--	--	--	--	--	--
80	0.6	NC	--	--	--	--	--	--
100	0.5	NC	--	--	--	--	--	--
150	0.5	NC	--	--	--	--	--	--
200	0.3	NC	--	--	--	--	--	--
250	0.2	NC	--	--	--	--	--	--
300	0.3	NC	--	--	--	--	--	--
400	0.3	NC	--	--	--	--	--	--
500	0.5	NC	--	--	--	--	--	--

---

*Not cloned.

Table 324

MOUSE LYMPHOMA FORWARD MUTATION ASSAY OF TRIALLATE IN THE ABSENCE OF METABOLIC ACTIVATION

Concentration (µg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Calculated Mutation Frequency (X 10^{-6})	Induced Mutation Frequency (X 10^{-6})
Solvent control	95.3	600	463	106	87.9	83.7	46	--
(1% DMSO)	104.7	600	591	128	112.1	117.4	43	--
Positive control	74.5	600	401	1013	76.1	56.7	505	461
(500 µg/ml EMS)	68.3	600	338	964	64.1	43.8	570	526
Triallate								
4.8	96.6	600	547	100	103.8	100.3	37	--
	101.0	600	565	91	107.2	108.3	32	--
12	86.3	600	556	83	105.5	91.0	30	--
	86.3	600	489	92	92.8	80.1	38	--
20	71.4	600	518	85	98.3	70.2	33	--
	75.0	600	419	70	79.5	59.6	33	--
32	44.9	600	518	82	98.3	44.1	32	--
	34.8	600	444	108	84.3	29.3	49	5
40	27.8	600	479	133	90.9	25.3	56	12
	27.8	600	428	124	81.2	22.6	58	14
44	23.4	600	463	104	87.9	20.6	45	1
	19.8	600	412	157	78.2	15.5	76	32
52	17.2	600	460	142	87.3	15.0	62	18
	15.6	600	411	182	78.0	12.2	89	45
60	11.3	600	415	215	78.7	8.9	104	60
	0.0	NC*	--	--	--	--	--	--
72	3.1	NC	--	--	--	--	--	--
	3.1	NC	--	--	--	--	--	--
80	18.7	NC	--	--	--	--	--	--
	6.0	NC	--	--	--	--	--	--

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*Not cloned.

Table 325

REPEAT MOUSE LYMPHOMA FORWARD MUTATION ASSAY OF TRIALLATE IN THE ABSENCE OF METABOLIC ACTIVATION

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Calculated Mutation Frequency (X 10^{-6})	Induced Mutation Frequency (X 10^{-6})
Solvent control	107.6	600	410	56	85.2	91.7	27	--
(1% DMSO)	92.4	600	552	73	114.8	106.0	26	--
Positive control	84.1	600	393	868	81.7	68.7	442	416
(500 μg/ml EMS)	84.7	600	391	918	81.3	68.9	470	444
Triallate								
4.8	112.1	600	559	79	116.2	130.3	28	2
	112.1	600	529	76	110.0	123.3	29	3
9	62.3	600	480	143	99.8	62.2	60	34
	101.6	600	565	91	117.5	119.3	32	6
12	125.4	600	543	81	112.9	141.6	30	4
	96.9	600	556	93	115.6	112.0	33	7
21	92.3	600	515	84	107.1	98.8	33	7
	96.8	600	493	139	102.5	99.2	56	30
27	84.7	600	549	80	114.1	96.7	29	3
	96.7	600	514	79	106.9	103.3	31	5
33	63.9	600	541	133	112.5	71.9	49	23
	84.1	600	563	91	117.0	98.4	32	6
39	52.7	600	522	66	108.5	57.2	25	--
	54.6	600	492	82	102.3	55.8	33	7
45	40.6	600	680*	112	141.4	57.4	33	7
	38.5	600	--	--	--	--	--	--
51	38.5	600	525	130	109.1	42.0	50	34
	32.7	600	548	119	113.9	37.3	43	17
60	15.6	600	419	120	87.1	13.6	57	31
	16.3	600	365	153	75.9	12.4	84	58

---

*Sample lost.

Table 326

MOUSE LYMPHOMA FORWARD MUTATION ASSAY OF TRIALLATE IN THE PRESENCE OF INDUCED METABOLIC ACTIVATION

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Calculated Mutation Frequency (X 10^{-6})	Induced Mutation Frequency (X 10^{-6})
Solvent control	101.1	600	520	162	102.8	103.9	62	--
(1% DMSO)	98.9	600	492	164	97.2	96.2	67	--
Positive control	58.5	600	326	517	64.4	37.7	317	253
(4.0 μg/ml 3-MCA)	52.9	600	357	528	70.6	37.3	296	232
Triallate								
0.5	102.5	600	578	129	114.2	117.1	45	--
	93.8	600	610	211	120.6	113.1	69	5
1	102.5	600	578	164	114.2	117.1	57	--
	92.9	600	625	218	123.5	114.7	70	6
3	90.2	600	630	185	124.5	112.3	59	--
	92.9	600	607	149	120.0	111.4	49	--
9	74.3	600	546	281	107.9	80.2	103	39
	93.0	600	447	245	88.3	82.2	110	46
12	78.5	600	537	276	106.1	83.3	103	39
	72.4	600	497	268	98.2	71.1	108	44
14	61.2	600	--*	--	--	--	--	--
	59.8	600	497	315	98.2	58.7	127	63
15	74.3	600	542	302	107.1	79.6	111	47
	67.3	600	541	281	106.9	71.9	104	40
18	38.2	600	513	288	101.4	38.7	112	48
	43.8	600	498	265	98.4	43.1	106	42
21	24.9	600	473	346	93.5	23.3	146	82
	22.6	600	428	280	84.6	19.1	131	67
24	17.2	600	328	322	64.8	11.1	196	132
	17.2	600	403	329	79.6	13.7	163	99

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*Sample lost.

Table 327

EFFECT OF TRIALLATE ON VIABILITY AND FERTILITY OF $y\ B/y^{+}Y$ MALES

Concentrations of Triallate/Day (%)	Viability (%)	Fertility (%)
Wide-Range Pretest		
0	100	100[†]
0.00000116[*]	100	100[†]
0.00000579[*]	100	100[†]
0.0000290[*]	100	90[†]
0.000145[*]	100	100[†]
0.000724[*]	100	80[†]
0.00276[*]	0	--
0.0138[*]	0	--
0.0905[*]	0	--
Narrow-Range Pretest		
0	100	90
0.0000313	90	100
0.0000625	100	100
0.000125	90	89
0.00025	100	90
0.0005	100	80
0.001	100	100
0.002	60	100
0.004	0	--

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[*] Average daily dose because of variations resulting from dilution errors.

[†] Judgment of fertility was based on the presence of third instar larvae because lack of O_2 killed all offspring in the pupal stage.

Table 328

NUMBERS OF SEX-LINKED RECESSIVE LETHAL MUTATIONS IN *DROSOPHILA MELANOGASTER* MALES FED TRIALLATE FOR 3 DAYS

Treatment	Experiment No.	No. of Lethals/Total Sample Size per Brood			Total Sample Size	Total No. Lethals
		A	B	C		
Solvent control (1% DMSO)	I	2/602	2/993	0/187	1782	4
	II	2/1183	3/427	0/248	1858	5
	III	4/2491	7/2263	4/413	5167	15[*]
	II and III	6/3674	10/2690	4/661	7025	20
	All	8/4276	12/3683	4/848	8807	24
Triallate (%)						
0.002	I	1/192	--[†]	0/16	208	1
0.001	II	7/2697	4/966	1/557	4220	12[‡]
0.001	III	5/1184	6/1833	3/1104	4121	14[§]
0.001	II and III	12/3881	10/2799	4/1661	8341	26
Positive control (0.01% EMS)	I	7/52	2/47	4/37	136	13[**]
	II	8/53	4/56	9/54	163	21[††]
	III	23/49	16/47	4/21	117	43[‡‡]
	II and III	31/102	20/103	13/75	280	64
	All	38/154	22/150	17/112	416	77

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[*] Includes 1 cluster of 2 and 1 cluster of 3; excludes 1 cluster of 7.

[†] No F_1 females were produced in this brood.

[‡] Includes 2 clusters of 2.

[§] Includes 1 cluster of 2.

[**] Includes 3 clusters of 2 and 1 cluster of 3.

[††] Includes 4 clusters of 2, 1 cluster of 3, 1 cluster of 4, and 1 cluster of 6.

[‡‡] Includes 1 cluster of 2, 2 clusters of 3, 2 clusters of 4, 1 cluster of 5, 2 clusters of 6, and 1 cluster of 10.

Table 329

SEX-LINKED RECESSIVE LETHAL MUTATION FREQUENCIES IN DROSOPHILA MELANOGASTER MALES FED TRIALLATE FOR 3 DAYS

Treatment	Experiment No.	Mutation Frequency (%) Brood A	B	C	All Broods
Solvent control (1% DMSO)	I	0.33	0.20	0	0.22
	II	0.17	0.70	0	0.27
	III	0.16	0.31	0.97	0.29
	II and III	0.16	0.37	0.61	0.28
	All	0.19	0.32	0.47	0.27
Triallate (%)					
0.002	I	0.52	--	0	0.48
0.001	II	0.26	0.41	0.18	0.28
0.001	III	0.42	0.33	0.27	0.34
0.001	II and III	0.31	0.36	0.24	0.31
Positive control (0.01% EMS)	I	13.5	4.3	10.8	9.6
	II	15.1	7.1	16.7	12.9
	III	46.9	34.0	19.0	36.8
	II and III	30.4	19.4	17.3	22.9
	All	24.7	14.7	15.2	18.5

Note: -- = No results.

Trichlorfon

S. typhimurium Plate Incorporation, E. coli WP-2, S. cerevisiae D3. Trichlorfon was previously tested and found to be positive, with and without activation, in these three systems (52).

S. cerevisiae D7 (Tables 330, 331). The first experiment tested concentrations ranging from 0.01 to 2.5%. In this experiment, mitotic crossing over and gene conversion were evident. In the second experiment, at concentrations ranging from 0.25 to 4.0%, toxicity was exhibited and there was a definite positive response for reverse mutation as well as for crossing-over and gene conversion. Thus, we conclude that trichlorfon was positive for all three parameters.

E. coli/B. subtilis Relative Toxicity. This compound was previously tested and found to be negative in both species (52). Reexamination of the data indicates that the results are more appropriately described as inconclusive.

S. typhimurium Relative Toxicity (Tables 332, 333). Trichlorfon was found to be positive in both strain pairs in this test.

SCE in CHO Cells (Tables 334-338). Trichlorfon was dissolved in 95% ethanol at a concentration of 500 mg/ml. At concentrations of 5000 μg/ml or less, trichlorfon did not precipitate in the culture medium or the metabolic activation mixture. Table 334 presents the results of the cytotoxicity test of trichlorfon in CHO cell cultures without metabolic activation. At concentrations $\geq$ 1000 μg/ml, trichlorfon was cytotoxic to CHO cells; at a concentration of 200 μg/ml, the cultures yielded primarily M_1 metaphases. At concentrations of 40 μg/ml and less, M_2 metaphases were produced. Table 335 presents the results of the cytotoxicity test of trichlorfon in CHO cell cultures with metabolic activation. At a concentration of 5000 μg/ml, primarily M_1 metaphases were produced; and at concentrations of 1000 μg/ml, primarily M_2 metaphases were produced.

Table 336 presents the SCE frequencies in CHO cells exposed to trichlorfon without metabolic activation. At a concentration of 80 μg/ml, the number of M_2 metaphase cells produced was insufficient for analysis. A one-way analysis of variance was conducted on SCE frequencies at the four lower concentrations of trichlorfon and in the negative controls. The variance between treatment groups was not significantly greater than the variance within treatment groups. However, there was a discrepancy between the observations of the two cytogeneticists; B's results indicated a concentration-related elevation in SCE frequencies, but A's results did not.

A third cytogeneticist evaluated these coded slides. Table 337 presents the observations of the three cytogeneticists. Like B, cytogeneticist C's observations also indicated concentration-related increases in SCE frequencies, which plateaued at 20 to 40 μg/ml trichlorfon. Analysis of the results revealed that the variance in SCEs/cell between treatment groups was significantly greater than the variance within treatment groups. We interpret these results to indicate that trichlorfon caused a concentration-related increase in the SCE frequencies of CHO cells without metabolic activation.

Table 338 presents the SCE frequencies in CHO cells exposed to trichlorfon with metabolic activation. The response appeared to be concentration-related, and the variance between treatment groups was significantly greater than the variance within treatment groups.

The test of trichlorfon with metabolic activation involved exposing CHO cells to a higher concentration range for a shorter time (125 to 2000 μg/ml for 2 hours) than that used in the test without metabolic activation (5 to 40 μg/ml for 21.5 hours). Therefore, direct comparisons are inappropriate. However, we conclude that trichlorfon does not require metabolic activation to increase the SCE frequencies in CHO cells.

L5178Y Mouse Lymphoma (Tables 339-343). Table 339 presents the results of concentration-range testing of trichlorfon in the absence of a metabolic activation system. The results showed that the range to use in mutagenesis tests was approximately 20 to 200 μg/ml (118.4% relative total growth at 20 μg/ml and 1.3% relative total growth at 200 μg/ml). Concentration-range test results of trichlorfon in the presence of the metabolic activation system (Table 340) showed that the approximate range to use in the mutagenesis assay was 200 to 400 μg/ml (85.7% relative total growth at 100 μg/ml and 2.2% relative total growth at 400 μg/ml).

Tables 341 and 342 present the results of mutagenesis testing of trichlorfon in the absence of the metabolic activation system. Trichlorfon was extremely toxic at concentrations greater than 140 μg/ml in the first assay, yielding relative total growth values of less than 6.0%. In the repeat assay, the high concentration of 200 μg/ml yielded an average relative total growth value of 21.2%. No significant depression in relative total growth was observed at 20 μg/ml in the first assay and at 1 μg/ml in the repeat assay. These concentrations yielded respective average relative total growth values of 95.5% and 91.5%. Over the tested range, a concentration-related increase in induced mutation frequency was observed in both assays. In the first assay, average mutation frequencies at least twice the spontaneous frequency were observed from 80 to 120 μg/ml, concentrations that yielded average relative total growth values of at least 10.0%. In the repeat assay, average mutation frequencies at least twice the spontaneous frequency were observed at 160 and 200 μg/ml, concentrations that yielded average relative total growth values of at least 10.0%.

Table 343 presents the results of mutagenesis tests of trichlorfon in the presence of the S-9 metabolic activation system. This compound was extremely toxic at concentrations greater than 300 μg/ml, yielding relative total growth values of less than 7.0%. The lowest concentration (100 μg/ml) yielded an average relative total growth value of 70.7%. Over the range tested, a concentration-related increase in induced mutation frequency was observed. Average mutation frequencies at least twice the spontaneous frequency were observed from 100 to 300 μg/ml (six concentrations), with average relative total growth values of at least 10.0%.

These combined results indicate a mutagenic effect of trichlorfon at the thymidine kinase locus in L5178Y mouse lymphoma cells, both with and without the metabolic activation system. In both cases trichlorfon increased the mutation frequency, in a concentration-related manner, above the frequencies observed with the solvent control. The presence of the metabolic activation system was not required to elicit the mutagenic effect.

UDS in WI-38 Cells. Trichlorfon was previously tested and found to be positive without activation and negative with activation (52).

Micronucleus Test (Tables 344, 345). The dosing and sacrifice schedules are shown in Table 344. The incidences of micronuclei in PCEs and the PCE-to-RBC ratios are given in Table 345. Trichlorfon was negative at all dose levels and sampling times with a β error of < 0.001 in each group. The DMSO vehicle control group was also negative at all times ($\beta < 0.01$), and the TMP positive control group was positive, with an α error of < 0.001. The mean PCE-to-RBC ratios were within normal limits (0.50-1.50) for all groups. Trichlorfon meets all of the criteria for a nonmutagenic decision in the micronucleus test except that of maximum tolerated dose. The low incidence of micronuclei and absence of an upward trend suggest that a higher dose may be negative also. We conclude that trichlorfon is negative in this test system.

Table 330

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D7

TRICHLORFON

Experiment 1

Compound	MA	% Conc.	Survivors		Crossing-Over				Gene Conversion		Reverse Mutation	
					Mitotic Recombinants		Total Aberrants		Convertants		Revertants	
			Cells Per ml ($\times 10^{-7}$)	%	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors	Per ml ($\times 10^{-1}$)	Per 10^6 Survivors	Per ml ($\times 10^{0}$)	Per 10^7 Survivors
Negative Control												
DMSO	-	10	4.4	100	0	0	6.5	15	140	32	24	5.5
	+	10	4.4	100	2.0	4.5	14	32	150	34	19	4.3
Positive Control												
1,2,3,4-Diepoxybutane	-	0.013	4.0	91	68	170	650	1600	1200	300	580	150
	+	0.013	3.5	80	53	150	520	1500	1500	430	700	200
Trichlorfon	-	0.01	4.4	100	2.0	4.5	9.0	20	190	43	13	3.0
	-	0.05	4.9	111	3.0	6.1	26	53	150	31	8.0	1.6
	-	0.1	4.7	107	0	0	13	28	180	38	4.0	0.85
	-	0.5	5.5	125	1.0	1.8	39	71	190	35	12	2.2
	-	1.0	6.1	139	3.0	4.9	25	41	240	39	20	3.3
	-	2.5	3.1	70	8.0	26	46	150	260	84	19	6.1
	+	0.01	5.4	123	0	0	20	37	160	30	16	3.0
	+	0.05	4.2	95	0	0	8.0	19	140	33	16	3.8
	+	0.1	5.8	132	0	0	15	26	130	22	24	4.1
	+	0.5	5.6	127	6.0	11	24	43	170	30	14	2.5
	+	1.0	4.1	93	6.0	15	45	110	180	44	4.0	0.98
	+	2.5	3.7	84	9.0	24	57	150	280	76	11	3.0

Table 331

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D7

TRICHLORFON

Experiment 2

			Survivors		Crossing-Over				Gene Conversion		Reverse Mutation	
					Mitotic Recombinants		Total Aberrants		Convertants		Revertants	
Compound	MA	% Conc.	Cells Per ml (× 10^{-7})	%	Per ml (× 10^{-3})	Per 10^5 Survivors	Per ml (× 10^{-3})	Per 10^5 Survivors	Per ml (× 10^{-1})	Per 10^6 Survivors	Per ml (× 10^0)	Per 10^7 Survivors
Negative Control												
DMSO	-	10	5.2	100	0	0	12	23	43	8.3	22	4.2
	+	10	5.7	100	2.5	4.4	12	21	54	9.5	22	3.9
Positive Control												
1,2,3,4-Diepoxybutane	-	0.013	4.6	88	97	210	810	1800	1700	370	350	76
	+	0.013	6.3	111	100	160	870	1400	1600	250	310	49
Trichlorfon	-	0.25	6.2	119	6.0	9.7	41	66	75	12	26	4.2
	-	0.5	6.6	127	16	24	55	83	130	20	30	4.5
	-	1.0	5.7	110	15	26	81	140	220	39	49	8.6
	-	2.5	2.0	38	20	100	160	800	340	170	47	24
	-	4.0	T*		T		T		T		T	
	+	0.25	6.0	105	1.0	1.7	33	55	81	14	21	3.5
	+	0.5	6.0	105	8.0	13	45	75	97	16	24	4.0
	+	1.0	6.0	105	10	17	86	140	190	32	26	4.3
	+	2.5	4.4	77	16	36	110	250	370	84	52	12
	+	4.0	T		T		T		T		T	

---

*T = toxic

Table 332

DIFFERENTIAL TOXICITY ASSAYS WITH SALMONELLA TYPHIMURIUM STRAINS

Experiment 1

Compound	Amount of Compound Applied to Disc	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		SL4525 (rec^+)		SL4700 (rec^-)		TA1978 ($uvrB^+$)		TA1538 ($uvrB^-$)	
Negative Control									
Kanamycin	**100.0 μg**	**14**	**13**	**13**	**14**	**13**	**13**	**14**	**14**
Positive Controls									
MMS[*]	**10.0 mg**	**47**	**46**	**68**	**65**				
4-NQO[†]	**2.0 mg**					**12**	**12**	**24**	**23**
Trichlorfon	1.0 mg	7	7	7	8	8	8	11	11
	2.0 mg	8	8	13	13	9	9	8	8

(Continued)

Table 333

DIFFERENTIAL TOXICITY ASSAYS WITH SALMONELLA TYPHIMURIUM STRAINS

Experiment 2

Compound	Amount of Compound Applied to Disc	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		SL4525 (rec$^+$)		SL4700 (rec$^-$)		TA1978 (uvrB$^+$)		TA1538 (uvrB$^-$)	
Negative Control									
Kanamycin	100.0 µg	15	14	16	15	17	17	15	16
Positive Controls									
MMS*	10.0 mg	45	40	72	74				
4-NQO†	2.0 mg					12	12	28	26
Trichlorfon	5.0 mg	10	9	11	11	10	10	9	11
	10.0 mg	10	10	13	14	12	11	16	17

Table 334

CYTOTOXICITY TEST OF CHINESE HAMSTER OVARY CELLS EXPOSED TO TRICHLORFON FOR 21.5 HOURS WITHOUT METABOLIC ACTIVATION

Treatment	Number of Divisions in Bromodeoxyuridine (BrdU)*
Negative control (0.95% ethanol)	M_2
Trichlorfon (μg/ml)	
8	M_2
40	M_2
200	$M_1 >> M_2$ †
1000	-- ‡
5000	-- ‡
Positive control (10^{-3} M ethyl methanesulfonate)	M_2

---

[*] M_1, one division in BrdU; M_2, two divisions in BrdU.

[†] The cell type to the left of the > sign was in the majority.

[‡] This culture not harvested because of excessive cytotoxicity.

Table 335

CYTOTOXICITY TEST OF CHINESE HAMSTER OVARY CELLS EXPOSED TO TRICHLORFON FOR 2 HOURS WITH METABOLIC ACTIVATION

Treatment	Number of Divisions in Bromodeoxyuridine (BrdU)*
Negative control (0.95% ethanol)	M_2
Trichlorfon (μg/ml)	
8	M_2
40	M_2
200	M_2
1000	M_2
5000	M_1
Positive control (10^{-3} M dimethylnitrosamine)	M_2

---

* M_1, one division in BrdU; M_2, two divisions in BrdU.

Table 336

SISTER CHROMATID EXCHANGE (SCE) FREQUENCIES IN CHINESE HAMSTER OVARY (CHO) CELLS EXPOSED TO TRICHLORFON FOR 21.5 HOURS WITHOUT METABOLIC ACTIVATION
(2 Cytogeneticists)

Treatment	Cyto-geneticist[*]	No. of SCEs	No. of Chromosomes	Chromosomes/Cell (Mean ± SD)	SCEs/Cell[†] (Mean ± SE)	SCEs/Chromosome[†] (Mean ± SE)
Negative control	A	315	493	19.72 ± 0.46	12.60 ± 0.71	0.639 ± 0.036
(0.95% ethanol)	B	243	478	19.12 ± 1.13	9.72 ± 0.62	0.508 ± 0.033
Trichlorfon (μg/ml)						
5	A	268	494	19.76 ± 1.05	10.72 ± 0.65	0.542 ± 0.033
	B	331	479	19.16 ± 0.55	13.24 ± 0.73	0.691 ± 0.038
10	A	350	490	19.60 ± 0.96	14.00 ± 0.75	0.714 ± 0.038
	B	311	473	18.92 ± 0.76	12.44 ± 0.71	0.658 ± 0.037
20	A	343	496	19.84 ± 0.47	13.72 ± 0.74	0.692 ± 0.037
	B	327	475	19.00 ± 0.58	13.08 ± 0.72	0.688 ± 0.038
40	A	327	490	19.60 ± 0.82	13.08 ± 0.72	0.667 ± 0.037
	B	420	478	19.12 ± 0.78	16.80 ± 0.82	0.879 ± 0.043
80		--[‡]	--	--	--	--
Positive control	A	1062	492	19.68 ± 1.07	42.48 ± 1.30	2.159 ± 0.066
(10^{-3} M ethyl methanesulfonate)	B	1144	486	19.44 ± 0.65	45.76 ± 1.35	2.354 ± 0.070

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[*] Each cytogeneticist analyzed 25 cells per sample.

[†] A one-way analysis of variance comparing the SCE frequencies in CHO cells exposed to trichlorfon and to the negative control indicated that the variance between treatment groups was not significantly greater than the variance within treatment groups.

[‡] Insufficient M_2 metaphases for analysis.

Table 337

SISTER CHROMATID EXCHANGE (SCE) FREQUENCIES IN CHINESE HAMSTER OVARY (CHO) CELLS EXPOSED TO TRICHLORFON FOR 21.5 HOURS WITHOUT METABOLIC ACTIVATION

(3 Cytogeneticists)

Treatment	Sample Number	Cyto-geneticist*	No. of SCEs	No. of Chromosomes	Chromosomes/Cell (Mean ± SD)	SCEs/Cell† (Mean ± SE)	SCEs/Chromosome† (Mean ± SE)
Negative control (0.95% ethanol)	1	A	315	493	19.72 ± 0.46	12.60 ± 0.71	0.639 ± 0.036
	1	C	279	474	18.96 ± 0.68	11.16 ± 0.67	0.589 ± 0.035
	2	B	243	478	19.12 ± 1.13	9.72 ± 0.62	0.508 ± 0.033
Trichlorfon (µg/ml)							
5	3	A	268	494	19.76 ± 1.05	10.72 ± 0.65	0.542 ± 0.033
	3	C	329	479	19.16 ± 1.07	13.16 ± 0.73	0.687 ± 0.038
	4	B	331	479	19.16 ± 0.55	13.24 ± 0.73	0.691 ± 0.038
10	5	B	311	473	18.92 ± 0.76	12.44 ± 0.71	0.658 ± 0.037
	5	C	305	408	19.20 ± 1.16	12.20 ± 0.70	0.635 ± 0.036
	6	A	350	490	19.60 ± 0.96	14.00 ± 0.75	0.714 ± 0.038
20	7	B	327	475	19.00 ± 0.58	13.08 ± 0.72	0.688 ± 0.038
	7	C	425	475	19.00 ± 1.12	17.00 ± 0.83	0.895 ± 0.043
	8	A	343	496	19.84 ± 0.47	13.72 ± 0.74	0.692 ± 0.037
	8	C	353	471	18.84 ± 1.11	14.12 ± 0.75	0.750 ± 0.040
40	9	B	420	478	19.12 ± 0.78	16.80 ± 0.82	0.879 ± 0.043
	9	C	394	483	19.32 ± 0.95	15.76 ± 0.79	0.816 ± 0.041
	10	A	327	490	19.60 ± 0.82	13.08 ± 0.72	0.667 ± 0.037
80			--‡	--	--	--	--
Positive control (10⁻⁷ M ethyl methanesulfonate)	13	A	1062	492	19.68 ± 1.07	42.48 ± 1.30	2.159 ± 0.066
	14	B	1144	486	19.44 ± 0.65	45.76 ± 1.35	2.354 ± 0.070

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[*] Each cytogeneticist analyzed 25 cells per sample.

[†] A one-way analysis of variance comparing the SCE frequencies in CHO cells exposed to trichlorfon and to the negative control indicated that the variance between treatment groups was significantly greater ($P < 0.05$) than the variance within treatment groups for SCEs/cell.

[‡] Insufficient M_2 metaphases for analysis.

Table 338

SISTER CHROMATID EXCHANGE (SCE) FREQUENCIES IN CHINESE HAMSTER OVARY (CHO) CELLS EXPOSED TO TRICHLORFON FOR 2 HOURS WITH METABOLIC ACTIVATION

Treatment	Cyto-geneticist[*]	No. of SCEs	No. of Chromosomes	Chromosomes/Cell (Mean ± SD)	SCEs/Cell[†] (Mean ± SE)	SCEs/Chromosome[†] (Mean ± SE)
Negative control (0.95% ethanol)	A	420	473	18.92 ± 1.15	16.80 ± 0.81	0.888 ± 0.045
	B	532	496	19.84 ± 0.90	21.28 ± 0.92	1.073 ± 0.047
Trichlorfon (μg/ml)						
125	A	415	482	19.28 ± 1.275	16.60 ± 0.81	0.861 ± 0.042
	B	576	494	19.76 ± 0.78	23.04 ± 0.96	1.166 ± 0.049
250	A	449	482	19.28 ± 1.40	17.96 ± 0.85	0.932 ± 0.044
	B	527	482	19.28 ± 1.02	21.08 ± 0.92	1.093 ± 0.048
500	A	505	514	19.77 ± 1.07	19.42 ± 0.86	0.982 ± 0.043
	B	587	483	19.32 ± 1.18	23.48 ± 0.97	1.215 ± 0.050
1000	A	683	490	19.60 ± 1.71	27.32 ± 1.05	1.394 ± 0.053
	B	661	497	19.88 ± 0.93	26.44 ± 1.03	1.330 ± 0.052
2000	A	746	468	18.72 ± 1.49	29.84 ± 1.09	1.594 ± 0.058
	B	694	485	19.40 ± 1.08	27.76 ± 1.05	1.431 ± 0.054
Positive control (10^{-3} M dimethylnitrosamine)	A	908	483	19.32 ± 1.44	36.32 ± 1.21	1.880 ± 0.062
	B	1022	484	19.36 ± 1.11	40.88 ± 1.28	2.132 ± 0.066

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[*] Each cytogeneticist analyzed 25 cells per sample; 26 cells were evaluated by cytogeneticist A for the 500 μg/ml treatment.

[†] A one-way analysis of variance comparing the SCE frequencies in CHO cells exposed to trichlorfon and to the negative control indicated that the variance between treatment groups was significantly greater ($P < 0.05$) than the variance within treatment groups.

Table 139

MOUSE LYMPHOMA FORWARD MUTATION CONCENTRATION-RANGE ASSAY OF TRICHLORFON IN THE ABSENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency (× 10^{-6})	Induced Mutation Frequency (× 10^{-6})
Solvent control	95.1	600	484	133	98.3	93.5	55	--
(1% DMSO)	104.9	600	501	124	101.7	106.7	50	--
Positive control	77.7	600	315	1016	64.0	49.7	645	592
(500 μg/ml EMS)	67.8	600	300	1006	60.9	41.3	671	618
Trichlorfon								
1	89.6	600	536	178	108.8	97.5	66	13
2	105.0	600	482	191	97.9	102.8	79	26
5	99.5	600	588	185	119.4	118.8	63	10
10	105.0	600	602	163	122.2	128.3	54	1
20	114.3	600	510	169	103.6	118.4	66	13
40	71.4	600	565	191	114.7	81.9	68	15
80	37.7	600	480	267	97.5	36.7	111	58
100	37.1	600	375	254	76.1	28.2	135	82
150	9.3	600	180	247	36.5	3.4	274	221
200	7.3	600	90	261	18.3	1.3	580	527
300	0.9	NC*	--	--	--	--	--	--
400	3.0	NC	--	--	--	--	--	--
500	0.5	NC	--	--	--	--	--	--
600	--†	--	--	--	--	--	--	--
700	0.5	NC	--	--	--	--	--	--
800	0.6	NC	--	--	--	--	--	--
900	0.5	NC	--	--	--	--	--	--
1000	0.5	NC	--	--	--	--	--	--

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*Not cloned.

†Sample lost.

Table 340

MOUSE LYMPHOMA FORWARD MUTATION CONCENTRATION-RANGE ASSAY OF TRICHLORFON IN THE PRESENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (µg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency (× 10^{-6})	Induced Mutation Frequency (× 10^{-6})
Solvent control (1 % DMSO)	--*	--	--	--	--	--	--	--
	100.0	600	481	274	100.0	100.0	114	--
Positive control (5 µg/ml 3-MCA)	44.6	600	263	668	54.7	24.4	508	394
	44.3	600	242	615	50.3	22.3	508	394
Trichlor								
1	106.2	600	539	257	112.1	119.0	95	--
2	106.2	600	518	235	107.7	114.4	91	--
5	106.2	600	606	267	126.0	133.8	88	--
10	102.5	600	537	274	111.6	114.4	102	--
20	102.5	600	580	258	120.6	123.6	89	--
40	105.1	600	522	303	108.5	114.1	116	2
80	93.5	600	571	355	118.7	111.0	124	10
100	88.6	600	465	365	96.7	85.7	157	43
150	98.8	600	494	421	102.7	101.5	170	56
200	57.3	600	453	493	94.2	54.0	218	104
300	33.4	600	311	457	64.7	21.6	294	180
400	10.0	600	108	442	22.5	2.2	819	705
500	8.6	600	59	435	12.3	1.1	1475	1361
600	3.3	NC†	--	--	--	--	--	--
700	2.5	NC	--	--	--	--	--	--
800	1.7	NC	--	--	--	--	--	--
900	1.7	NC	--	--	--	--	--	--
1000	2.1	NC	--	--	--	--	--	--

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[*] Sample lost.

[†] Not cloned.

Table 341

MOUSE LYMPHOMA FORWARD MUTATION ASSAY OF TRICHLORFON IN THE ABSENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (µg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency (× 10^{-6})	Induced Mutation Frequency (× 10^{-6})
Solvent control (1% DMSO)	93.0	600	501	112	97.6	90.7	45	--
	107.0	600	526	92	102.4	109.6	35	--
Positive control (500 µg/ml EMS)	75.6	600	385	809	75.0	56.7	420	380
	70.1	600	361	972	70.3	49.3	539	499
Trichlorfon								
20	93.0	600	550	102	107.1	99.6	37	--
	85.4	600	549	134	106.9	91.3	49	9
40	64.7	600	505	212	98.3	63.6	84	44
	60.3	600	431	194	83.9	50.6	90	50
60	64.7	600	514	170	100.1	64.8	66	26
	65.0	600	518	202	100.9	65.6	78	38
80	44.8	600	472	251	91.9	41.2	106	66
	36.2	600	474	266	92.3	33.4	112	72
100	35.4	600	491	326	95.6	33.8	133	93
	30.3	600	403	322	78.5	23.8	160	120
120	14.8	600	327	375	63.7	9.4	229	189
	26.2	600	316	322	61.5	16.1	204	164
140	18.1	600	258	372	50.2	9.1	288	248
	18.4	600	265	388	51.6	9.5	293	253
160	14.8	600	206	370	40.1	5.9	359	319
	13.5	600	210	417	40.9	5.5	397	357
180	9.4	600	122	330	23.8	2.2	541	501
	9.1	600	110	259	21.4	1.9	471	431
200	9.5	600	97	284	18.9	1.8	586	546
	14.4	600	204	356	39.7	5.7	349	309

Table 342

REPEAT MOUSE LYMPHOMA FORWARD MUTATION ASSAY OF TRICHLORFON IN THE ABSENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency (× 10⁻⁶)	Induced Mutation Frequency (× 10⁻⁶)
Solvent control (1% DMSO)	109.6	600	527	245	106.0	116.2	93	--
	90.4	600	467	146	94.0	84.9	63	--
Positive control (500 μg/ml EMS)	59.1	600	302	944	60.8	35.9	625	547
	61.0	600	258	839	51.9	31.7	650	572
Trichlorfon								
10	87.0	600	566	189	113.9	99.1	67	--
	87.0	600	479	154	96.4	83.8	64	--
30	83.2	600	478	158	96.2	80.0	66	--
	83.2	600	475	178	95.6	79.5	75	--
60	73.9	600	436	163	87.7	64.8	75	--
	68.4	600	413	167	83.1	56.8	81	3
70	70.9	600	463	163	93.2	66.0	70	--
	65.8	600	437	186	87.9	57.9	85	7
80	63.8	600	480	151	96.6	61.6	63	--
	68.9	600	416	173	83.7	57.7	83	5
100	57.3	600	431	164	86.7	49.7	76	--
	55.1	600	509	181	102.4	56.4	71	--
120	51.9	600	471	199	94.8	49.2	85	7
	50.1	600	480	220	96.6	48.4	92	14
140	55.0	600	500	220	100.6	55.3	88	10
	48.3	600	422	235	84.9	41.0	111	33
160	33.3	600	369	286	74.2	24.7	155	77
	14.2	600	259	409	52.1	7.4	316	238
200	29.6	600	303	329	61.0	18.0	217	139
	31.7	600	381	287	76.7	24.3	151	73

Table 343

MOUSE LYMPHOMA FORWARD MUTATION ASSAY OF TRICHLORFON IN THE PRESENCE OF A METABOLIC ACTIVATION SYSTEM

Concentration (μg/ml)	Relative Suspension Growth (%)	Total Cells Cloned for VC	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutation Frequency (× 10^{-6})	Induced Mutation Frequency (× 10^{-6})
Solvent control (1% DMSO)	103.1	600	458	203	100.8	103.9	89	--
	96.9	600	451	162	99.2	96.2	72	--
Positive control (7 μg/ml 3-MCA)	29.2	600	131	323	28.8	8.4	493	412
	33.6	600	163	343	35.9	12.1	421	340
Trichlorfon								
100	78.7	600	411	350	90.4	71.2	170	89
	75.0	600	425	338	93.5	70.1	159	78
150	59.7	600	359	342	79.0	47.2	191	110
	63.2	600	365	375	80.3	50.8	205	124
200	42.3	600	307	377	67.5	28.6	246	165
	49.1	600	279	325	61.4	30.1	233	152
225	45.6	600	238	360	52.4	23.9	303	222
	48.4	600	277	357	60.9	29.5	258	177
250	40.9	600	336	353	73.9	30.2	210	129
	30.7	600	237	317	52.1	16.0	268	187
300	31.2	600	189	333	41.6	13.0	352	271
	29.3	600	160	338	35.2	10.3	423	342
350	17.4	600	104	249	22.9	4.0	479	398
	22.3	600	139	248	30.6	6.8	357	276
375	14.7	600	100	247	22.0	3.2	494	413
	14.5	600	83	252	18.3	2.6	607	526
400	15.6	600	94	254	20.7	3.2	540	459
	12.5	600	100	236	22.0	3.9	472	391
500	10.1	600	55	444	12.1	1.2	1615	1534
	14.7	600	76	247	16.7	2.5	650	569

Table 344

DOSING AND SACRIFICE SCHEDULE

Compound	No. of Animals	Dose		Mean Body Wt Change (g)	Number Sacrificed			No. of Fatalities
		0 Hr	24 Hr		48 Hr	72 Hr	96 Hr	
DMSO								
Vehicle control	24*	5 ml/kg	5 ml/kg	-1.2	8	7	8	1
TMP								
Positive control	8*	1 g/kg	1 g/kg	-2.4	8	-	-	0
Trichlorfon								
Low dose	24	100 mg/kg	100 mg/kg	-1.0	8	8	8	0
Mid dose	24	200 mg/kg	200 mg/kg	-0.8	8	8	8	0
High dose	24	400 mg/kg	400 mg/kg	-0.9	8	8	8	0

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* The control groups are the same as those reported with rotenone, which was tested at the same time.

Table 345

INCIDENCE OF MICRONUCLEATED POLYCHROMATOPHILIC ERYTHROCYTES*

(500 PCE scored per animal)

Group	48 Hours			72 Hours			96 Hours		
	Animal No.	PCE with MN	PCE/RBC	Animal No.	PCE with MN	PCE/RBC	Animal No.	PCE with MN	PCE/RBC
DMSO 2 x 5 ml/kg Vehicle Control	101ø	1	1.30	103ø	1	1.98	102ø	0	0.70
	101r	0	1.49	103r	1	1.83	102r	1	1.00
	101ℓ	2	1.09	103ℓ	0	1.61	102ℓ	0	1.09
	101b	0	0.88	103b	2	0.59	102b	0	0.81
	104ø	1	0.81	106r	1	1.28	105ø	1	0.98
	104r	1	0.75	106ℓ	1	1.47	105r	1	1.56
	104ℓ	1	0.92	106b	1	1.23	105ℓ	1	1.24
	104b	0	1.47				105b	0	1.67
Total		6†			7‡			4†	
Mean			1.09			1.43			1.13
Trichlorfon 2 x 100 mg/kg	129ø	0	1.05	128ø	2	0.80	130ø	1	0.98
	129r	0	1.25	128r	1	1.56	130r	1	1.20
	129ℓ	0	1.05	128ℓ	0	2.27	130ℓ	0	1.94
	129b	1	0.84	128b	0	0.87	130b	0	1.78
	132ø	2	0.78	133ø	0	1.52	131ø	1	1.65
	132r	0	1.25	133r	0	1.03	131r	0	1.06
	132ℓ	0	1.27	133ℓ	2	1.48	131ℓ	0	0.98
	132b	0	0.87	133b	1	0.50	131b	2	0.63
Total		3†			6†			5†	
Mean			1.05			1.25			1.28
Trichlorfon 2 x 200 mg/kg	135ø	1	0.52	136ø	0	0.94	134ø	1	1.16
	135r	0	0.62	136r	0	1.15	134r	0	0.79
	135ℓ	0	0.63	136ℓ	0	1.69	134ℓ	0	1.25
	135b	0	1.27	136b	1	0.93	134b	0	1.29
	139ø	0	1.44	137ø	0	0.85	138ø	0	1.90
	139r	1	1.03	137r	0	1.97	138r	0	1.27
	139ℓ	2	0.73	137ℓ	1	0.81	138ℓ	0	1.30
	139b	0	1.33	137b	1	0.59	138b	2	1.37
Total		4†			3†			3†	
Mean			0.95			1.12			1.29

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* PCE = Polychromatophilic erythrocytes, MN = micronucleus, RBC = mature erythrocytes.

† $\beta \leq 0.001$.

‡ $\beta = 0.01$.

Table 345 (Continued)

Group	48 Hours			72 Hours			96 Hours		
	Animal No.	PCE with MN	PCE/RBC	Animal No.	PCE with MN	PCE/RBC	Animal No.	PCE with MN	PCE/RBC
Trichlorfon 2 x 400 mg/kg	141ø	0	1.15	142ø	0	0.62	140ø	0	1.33
	141r	0	1.18	142r	0	1.01	140r	0	1.01
	141ℓ	0	1.10	142ℓ	0	1.12	140ℓ	1	0.82
	141b	0	1.16	142b	0	0.60	140b	0	0.59
	144ø	0	1.88	145ø	1	1.34	143ø	1	0.92
	144r	1	1.11	145r	1	1.64	143r	1	1.27
	144ℓ	1	1.00	145ℓ	1	0.90	143ℓ	0	0.77
	144b	0	1.45	145b	0	0.75	143b	0	0.91
Total		2†			3†			3†	
Mean			1.25			1.00			1.08
TMP 2 x 1 g/kg	107ø	4	0.92						
	107r	18	0.62						
	107ℓ	2	0.92						
	107b	5	1.51						
	108ø	3	0.71						
	108r	4	1.32						
	108ℓ	0	1.01						
	108b	2	0.90						
Total		38§							
Mean			0.99						

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† $\beta \leq 0.001$.
§ $\alpha < 0.001$.

Trifluralin

S. typhimurium Plate Incorporation (Table 346). Trifluralin was previously tested and found to be negative with and without activation in strains TA100, TA1535, TA1537 and TA1538 at concentrations up to 1000 µg/plate (51). It was retested in these four strains and in strain TA98 at concentrations up to 5000 µg/plate, and was negative with and without activation.

E. coli WP-2 (Table 346). This compound was previously tested and found to be negative with and without activation (51). Upon retesting at higher concentrations under the present contract, similar findings were obtained.

S. cerevisiae D3, E. coli/B. subtilis Relative Toxicity, UDS in WI-38 Cells. Trifluralin was previously tested in these three systems and was negative in all cases (51).

Table 346

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND E. COLI

TRIFLURALIN
Retest

Compound	Metabolic Activation	Compound Added (µg/plate)	Salmonella typhimurium Histidine Revertants per Plate										E. coli Tryptophan Revertants per Plate	
			TA1535		TA1537		TA1538		TA98		TA100		WP2	
Negative Control														
DMSO	-		14	15*	5	10	22	C	22	18	99	93	25	31
	+		23	C	5	5	19	18	25	28	87	99	52	40
Positive Controls														
2-Anthramine	-	1.0					12		17		87			
	+	1.0					337		289		495			
	-	2.5	13		6									
	+	2.5	322		149									
	-	10.0											18	
	+	10.0											640	
Sodium Azide	-	0.5	300											
	-	1.0									598			
9-Aminoacridine	-	50.0			209									
Nitrofluorene	-	5.0					1038	734						
AF2	-	0.1											807	
Trifluralin*	-	50.0	19		10		6		27		77		23	
	-	100.0	15		6		14		19		106		27	
	-	500.0	18		7		10		21		95		23	
	-	1,000.0	25		6		13		18		121		22	
	-	2,500.0	14		2		15		18		112		19	
	-	5,000.0	26		8		19		21		127		24	
	+†	50.0	10		10		20		31		123		40	
	+	100.0	11		3		18		23		149		35	
	+	500.0	15		3		13		27		146		38	
	+	1,000.0	15		7		13		23		119		57	
	+	2,500.0	12		4		14		28		121		48	
	+	5,000.0	9		6		18		26		121		37	

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*C, contaminated.

Vegadex

S. typhimurium Plate Incorporation, E. coli WP-2 (Tables 347, 348). When tested over a dose range of 0.01 to 2.5 µℓ/plate (~ 0.01 to 2.5 mg/plate) with the rat liver metabolic activation system, vegadex caused a dose-related increase in histidine-independent revertants with strains TA1535 and TA100 and only a slight increase with TA98 (Table 347). When vegadex was retested over a lower dose range, 0.001 to 0.5 µℓ/plate (~ 0.001 to 0.5 mg/plate), a similar mutagenic response was observed (Table 348). Toxicity was observed with the S typhimurium strains at 0.5 to 1.0 µℓ of vegadex per plate (~ 0.05 to 1.0 mg/plate). Vegadex was neither toxic nor mutagenic in the E. coli WP2 assay. The increase in mutagenic activity in strains TA1535 and TA100 when metabolic activation was provided indicates that vegadex is a base-pair substitution mutagen. We conclude that vegadex induces base-pair substitutions in S. typhimurium strains TA1535 and TA100 when activated by rat liver homogenate. A weak mutagenic response may have occurred with TA98. Vegadex was not mutagenic in two other Salmonella strains (TA1537 and TA1538) or in E. coli WP2.

S. cerevisiae D3 (Tables 349, 350). No recombinogenic activity was observed in this assay with vegadex. The pesticide was tested twice at doses of 0.1 to 4.0%. Survival was reduced to approximately 50% at the 2.0% concentration with and without metabolic activation.

E. coli/B. subtilis Relative Toxicity (Table 351). Vegadex was equally toxic to both repair-proficient and -deficient strains, thus it was negative in this assay. The zones of inhibition were very small, indicating that vegadex either is not very toxic to any of the four indicator bacterial strains or does not diffuse readily from the filter paper disc into the agar.

Table 347

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

VEGADEX

Experiment 1

Compound	Metabolic Activation	Amount of Compound Added per Plate	Salmonella typhimurium Histidine Revertants per Plate					E. coli Tryptophan Revertants per Plate
			TA1535	TA1537	TA1538	TA98	TA100	WP2
Negative control	-		25	9	13	25	125	44
	+		12	8	24	35	110	48
Positive controls								
9-Aminoacridine	-	100 µg		884				
2-Nitrofluorene	-	5.0			597			
2-Anthramine	-	1.0			11			
	+	1.0			233			
	+	2.5	345			1321	1837	78
Vegadex	-	0.01 µl	42	3	15	25	136	39
	-	0.05	49	4	15	29	178	25
	-	0.10	33	6	12	21	154	31
	-	0.50	32	1	T*	9	82	33
	-	1.00	T	T	T	T	T	35
	-	2.50	T	T	T	T	T	31
	+	0.01	54	6	25	37	254	46
	+	0.05	189	15	15	43	549	54
	+	0.10	236	8	21	61	748	39
	+	0.50	T	2	10	T	T	30
	+	1.00	T	T	T	T	T	31
	+	2.50	T	T	T	T	T	34

---

* T, toxic.

Table 348

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

VEGADEX

Experiment 2

Compound	Metabolic Activation	Amount of Compound Added per Plate	Salmonella typhimurium Average Histidine Revertants per Plate					E. coli Tryptophan Revertants per Plate
			TA1535	TA1537	TA1533	TA98	TA100	WP2
Negative control	-		23	14	14	23	111	27
	+		14	8	22	25	100	33
Positive controls								
2-Anthramine	-	1.0 µg			15	32	116	
	+	1.0			186	111	268	
	-	2.5	26	9				
	+	2.5	165	80				
	-	10						30
	+	10						105
9-Aminoacridine	-	50		312				
Sodium azide	-	0.5	208				257	
2-Nitrofluorene	-	5.0			586	438		
AF2	-	0.1						706
Vegadex	-	0.001 µl	18	3	13	21	86	28
	-	0.005	15	8	17	19	113	20
	-	0.010	14	12	19	28	117	28
	-	0.050	24	8	15	23	112	27
	-	0.100	15	7	13	26	104	27
	-	0.250	17	4	23	24	83	35
	-	0.500	21	3	T*	14	T	27
	+	0.001	15	9	23	31	101	37
	+	0.005	35	10	19	25	167	40
	+	0.010	65	7	17	33	277	38
	+	0.050	198	9	25	45	685	54
	+	0.100	366	7	12	57	1123	37
	+	0.250	471	6	15	35	743	46
	+	0.500	426	4	T	26	T	52

---

* T, toxic.

Table 349

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

VEGADEX

Experiment 1

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors Cells per ml (x 10^{-7})	Survivors Percent	Mitotic Recombinants Per ml (x 10^{-3})	Mitotic Recombinants Per 10^5 Survivors
Negative control	-		9.5	100	6.5	6.8
	+		9.7	100	9.5	9.8
Positive control 1,2,3,4-Diepoxybutane	-	0.025	8.9	94	995.0	1100
	+	0.025	6.9	71	790.0	1100
Vegadex	-	1.0	7.5	79	6.0	8.0
	-	2.0	5.0	53	3.0	6.0
	-	4.0	7.3	77	9.0	12
	-	5.0	7.1	75	10.0	14
	+	1.0	7.0	72	4.0	5.7
	+	2.0	5.4	56	7.0	13.
	+	4.0	8.0	82	11.0	14
	+	5.0	6.6	68	8.0	12

Table 350

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

VEGADEX

Experiment 2

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml (x 10^{-7})	Percent	Per ml (x 10^{-3})	Per 10^5 Survivors
Negative control	-		8.9	100	4.5	5.1
	+		7.1	100	5.0	7.0
Positive control 1,2,3,4-Diepoxybutane	-	0.025	10.4	117	530.0	510
	+	0.025	6.7	94	767.5	1100
Vegadex	-	1.0	8.4	94	11.0	13
	-	2.0	8.7	98	5.0	5.8
	-	3.0	9.8	110	10.0	10
	-	4.0	10.0	112	8.0	8.0
	-	5.0	9.5	107	5.0	5.3
	+	1.0	9.2	130	8.0	8.7
	+	2.0	7.8	110	4.0	5.1
	+	3.0	9.5	134	12.0	
	+	4.0	9.4	132	8.0	8.5
	+	5.0	9.4	132	11.0	12

Table 351

DIFFERENTIAL TOXICITY TO REPAIR-PROFICIENT AND -DEFICIENT BACTERIA

Compound	Milligrams of Compound Applied to Disc	Average Diameter of Zone of Inhibition* (in mm, disc = 6 mm) E. coli W3110	E. coli p3478	B. subtilis H17	B. subtilis M45
Negative control Ampicillin	0.1	19	19	26	28
Positive control Methylmethanesulfonate	1.0	27	34	15	48
Vegadex	10	7	6	8	8
	20	6	6	7	7

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* Average of three plates.

Industrial Chemicals

Acetonitrile

S. typhimurium Plate Incorporation, E. coli WP-2 (Tables 352-355). Acetonitrile was tested on separate days in four experiments over a range of 0.05 to 500 μℓ/plate (~ 0.04 to 400 mg/plate, density ≈ 0.8). Toxicity, denoted by the appearance of pinpoint colonies on the background, was observed at the highest dose in all cases except S. typhimurium strain TA1537. No reproducible dose-related increase in the number of revertants was observed with acetonitrile in either test system.

S. cerevisiae D3 (Tables 356-362). This compound was tested on separate days in seven experiments over a range of 0.1 to 10 percent using either water or DSMO as the diluent. Toxicity occurred at concentrations of 9 or 10%. Although increases in revertants were seen sporadically, no reproducible significant dose-related effect was apparent. Thus, on the basis of these experiments, we conclude that acetonitrile is negative in this system.

E. coli/B. subtilis Relative Toxicity (Tables 363-365). Acetonitrile was tested in five experiments over a range of 10 to 50 μℓ/plate. It caused no zone of inhibition of growth of either the repair-proficient or the repair-deficient bacteria at 10 or at 20 μℓ/plate, nor did it cause any differential inhibition between the repair-proficient and repair-deficient strains at 25 or 50 μℓ/plate. Thus, we conclude that acetonitrile was negative in this assay.

Table 352

IN VITRO ASSAYS WITH *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI* WP-2
ACETONITRILE
Experiment 1

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate										Tryptophan Revertants per Plate	
			TA1535		TA1537		TA1538		TA98		TA100		WP2	
Negative control	-		36	18	6	8	15	19	31	28	116	115	32	27
	+		10	7	12	11	24	27	39	43	132	125	35	34
Positive controls														
Sodium azide	-	1.0 μg	333	384							347	356		
9-Aminoacridine	-	50.0			316	254								
2-Nitrofluorene	-	5.0					1067	1044	664	679				
AF2	-	0.1											1428	1416
2-Anthramine	-	1.0					20	26	28	30	165	154		
	+	1.0					442	330	480	579	751	882		
	-	2.5	26	16	9	13								
	+	2.5	397	396	171	224								
	-	10.0											37	27
	+	10.0											689	967
Acetonitrile	-	0.05 μl	20	19	6	8	13	12	23	19	125	120	26	42
	-	0.1	18	15	7	10	13	14	24	31	113	128	33	31
	-	0.5	24	28	4	4	12	8	30	19	117	102	36	36
	-	1.0	18	20	8	5	10	8	27	29	123	122	33	36
	-	5.0	24	18	7	5	9	9	31	28	136	123	41	40
	-	10.0	20	18	3	6	8	9	26	26	102	110	41	42
	+	0.05	10	15	15	12	21	18	31	43	128	127	38	44
	+	0.1	14	18	12	14	25	24	31	31	111	128	37	38
	+	0.5	10	11	8	10	19	16	40	37	100	125	38	31
	+	1.0	11	12	13	7	17	19	30	32	87	139	39	49
	+	5.0	14	19	9	12	20	18	37	37	93	90	41	43
	+	10.0	7	13	12	20	13	29	27	25	104	100	33	40

Table 353

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI WP-2
ACETONITRILE
Experiment 2

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate										Tryptophan Revertants per Plate	
			TA1535		TA1537		TA1538		TA98		TA100		WP2	
Negative control	-		38	37	4	5	16	16	27	22	137	120	47	52
	+		15	15	13	17	21	25	42	43	122	116	46	43
Positive controls														
Sodium azide	-	0.5 µg	341	383										
	-	1.0									649	652		
9-Aminoacridine	-	50.0			134	225								
2-Nitrofluorene	-	5.0					617	652	393	430				
AF2	-	0.1											1399	1434
2-Anthramine	-	1.0					16	25	28	30	134	109		
	+	1.0					379	385	406	625	735	662		
	-	2.5	44	28	13	8								
	+	2.5	219	275	140	152								
	-	10.0											41	40
	+	10.0											921	880
Acetonitrile	-	0.05 µl	38	32	8	7	12	11	21	21	120	123	50	40
	-	0.1	35	20	8	9	18	17	23	25	125	102	41	34
	-	0.5	25	29	7	9	20	12	30	25	130	108	42	30
	-	1.0	31	45	9	7	10	16	26	22	113	110	47	46
	-	5.0	20	40	5	7	15	16	25	19	110	120	39	35
	-	10.0	37	29	5	10	15	12	28	22	107	102	47	45
	+	0.05	7	9	19	18	24	30	37	33	99	105	50	49
	+	0.1	8	10	19	18	28	26	29	33	113	100	47	51
	+	0.5	8	9	15	16	25	27	37	32	104	103	50	48
	+	1.0	10	13	13	19	31	30	44	29	100	115	49	51
	+	5.0	14	6	21	20	19	27	36	30	113	125	48	52
	+	10.0	13	7	12	14	11	28	42	33	103	102	52	49

Table 354

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI WP-2

ACETONITRILE

Experiment 3

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate TA1535		TA1537		TA1538		TA98		TA100		Tryptophan Revertants per Plate WP2	
Negative Control														
H₂O	-	500.0 µl	16	20	6	13	19	18	26	21	102	100	37	31
	+	500.0	18	13	15	13	21	16	41	28	96	113	52	54
Positive Controls														
Sodium Azide	-	0.5 µg	125	123										
	-	1.0									386	366		
9-Aminoacridine	-	50.0			194	88								
2-Nitrofluorene	-	5.0					737	780	370	437				
AF-2	-	0.1											1375	1434
2-Anthramine	-	1.0					25	20	28	42	110	111		
	+	1.0					338	430	246	287	390	420		
	-	2.5	20	17	9	9								
	+	2.5	160	153	127	131								
	-	10.0											36	40
	+	10.0											375	370
Acetonitrile	-	10.0 µl	25	17	7	13	10	9	28	18	117	115	52	30
	-	25.0	20	21	11	16	9	9	31	31	113	126	38	34
	-	50.0	28	17	12	13	17	10	18	33	91	103	31	37
	-	100.0	18	19	16	17	19	18	21	25	102	116	32	39
	-	250.0	17	21	7	6	16	21	25	18	88	90	38	23
	-	500.0	PP*	PP	10	4	PP	PP	PP	PP	77	77	26	PP
	+	10.0 µl	13	10	15	13	19	31	37	33	123	110	65	29
	+	25.0	14	13	17	13	21	30	40	36	111	111	50	30
	+	50.0	14	18	14	13	21	13	41	42	123	103	34	45
	+	100.0	10	8	15	17	31	29	33	42	130	110	41	40
	+	250.0	5	10	13	7	19	20	30	39	93	92	57	36
	+	500.0	PP	PP	7	5	21	18	25	37	72	80	32	43

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*PP = Pinpoint colonies on background.

Table 355

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI WP-2

ACETONITRILE

Experiment 4

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate										Tryptophan Revertants per Plate	
			TA1535		TA1537		TA1538		TA98		TA100		WP2	
Negative Conrol														
H_2O	-	500.0 µl	32	28	13	21	13	18	32	29	134	113	21	28
	+	500.0	19	16	28	26	25	18	42	52	149	152	54	49
Positive Controls														
Sodium Azide	-	0.5 µg	233	247										
	-	1.0									347	349		
9-Aminoacridine	-	50.0			135	150								
2-Nitrofluorene	-	5.0					768	821	446	427				
AF-2	-	0.1											1294	1363
2-Anthramine	-	1.0					18	17	20	26	131	142		
	+	1.0					187	238	245	260	592	493		
	-	2.5	26	26	33	18								
	+	2.5	195	207	149	125								
	-	10.0											31	32
	+	10.0											596	542
Acetonitrile	-	10.0 µl	34	32	21	17	14	13	18	29	142	113	32	27
	-	25.0	32	27	15	21	12	14	27	21	110	136	40	40
	-	50.0	24	39	15	15	14	18	28	28	124	135	44	33
	-	100.0	26	27	17	12	7	10	24	19	99	137	42	41
	-	250.0	30	39	15	7	14	14	20	15	102	111	33	24
	-	500.0	28	48	7	6	18	14	PP	PP	100	PP	18	16
	+	10.0 µl	16	12	18	13	29	24	30	40	91	115	40	38
	+	25.0	12	18	16	8	25	25	39	39	100	123	49	49
	+	50.0	12	18	12	12	17	19	36	38	113	101	41	51
	+	100.0	12	17	18	9	17	24	31	36	108	101	61	54
	+	250.0	14	15	9	10	20	18	18	29	114	103	38	45
	+	500.0	10	18	9	8	PP	PP	PP	PP	PP	PP	18	27

---

*PP = Pinpoint colonies on background.

Table 356

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
ACETONITRILE
Experiment 1

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors: Cells per ml ($x 10^{-7}$)	Survivors: Percent	Mitotic Recombinants: Per ml ($x 10^{-3}$)	Mitotic Recombinants: Per 10^5 Survivors
Negative control						
DMSO	-		5.8	100	7.0	12
	+		5.9	100	4.0	6.8
Positive control						
1,2,3,4-Diepoxybutane	-	0.025	7.4	128	620.0	840
	+	0.025	3.5	59	343.0	980
Acetonitrile	-	0.1	8.0	138	3.0	3.8
	-	0.5	8.5	147	2.0	2.4
	-	1.0	8.5	147	2.0	2.4
	-	5.0	10.0	172	2.0	2.0
	+	0.1	C*	C	C	C
	+	0.5	8.4	142	8.0	9.5
	+	1.0	9.5	161	5.0	5.3
	+	5.0	9.6	163	1.0	1.0

---

*C, contaminated.

Table 357

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
ACETONITRILE
Experiment 2

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($x\ 10^{-7}$)	Percent	Per ml ($x\ 10^{-3}$)	Per 10^5 Survivors
Negative control						
DMSO	-		8.6	100	5.5	6.4
	+		9.4	100	4.5	4.8
Positive control						
1,2,3,4-Diepoxybutane	-	0.025	5.0	58	580	1200
	+	0.025	6.5	69	928	1400
Acetonitrile	-	1	6.4	74	3.0	4.7
	-	2	7.1	83	5.0	7.0
	-	3	6.2	72	7.0	11
	-	4	7.4	86	6.0	8.1
	-	5	6.3	73	4.0	6.4
	+	1	6.2	66	5.0	8.1
	+	2	5.2	55	6.0	12
	+	3	7.7	82	9.0	12
	+	4	7.7	82	6.0	7.8
	+	5	6.7	71	4.0	6.0

Table 358

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
ACETONITRILE
Experiment 3

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml $(x\ 10^{-7})$	Percent	Per ml $(x\ 10^{-3})$	Per 10^5 Survivors
Negative Control						
H_2O	-		8.5	100	6.5	7.7
	+		8.5	100	4.5	5.3
Positive Control						
1,2,3,4-Diepoxybutane	-	0.025	7.8	92	600	770
	+	0.025	9.4	111	600	640
Acetonitrile	-	1.0	11.0	129	4.0	3.6
	-	2.0	8.0	94	4.0	5.0
	-	3.0	10.0	118	6.0	6.0
	-	4.0	11.0	129	3.0	2.7
	-	5.0	7.9	93	7.0	8.9
	+	1.0	11.0	129	4.0	3.6
	+	2.0	13.0	153	6.0	4.6
	+	3.0	9.4	111	5.0	5.3
	+	4.0	9.5	112	7.0	7.4
	+	5.0	8.4	99	5.0	6.0

Table 359

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
ACETONITRILE
Experiment 4

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml (x 10^{-7})	Percent	Per ml (x 10^{-3})	Per 10^5 Survivors
Negative Control						
H_2O	-		7.6	100	1.5	2.0
	+		7.7	100	4.0	5.2
Positive Control						
1,2,3,4-Diepoxybutane	-	0.025	7.8	103	650	830
	+	0.025	8.9	117	280	310
Acetonitrile	-	5.0	5.6	74	1.0	1.8
	-	6.0	6.6	87	5.0	7.6
	-	7.0	7.3	96	6.0	8.2
	-	8.0	6.6	87	1.0	1.5
	-	9.0	5.5	72	9.0	16
	-	10.0	T*	T	T	T
	+	5.0	8.7	113	4.0	4.6
	+	6.0	6.9	90	5.0	7.3
	+	7.0	7.0	91	9.0	13
	+	8.0	4.8	62	2.0	4.2
	+	9.0	5.6	73	18	32
	+	10.0	T	T	T	T

---

*T = toxic.

Table 360

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
ACETONITRILE
Experiment 5

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative Control						
H_2O	-		7.6	100	6.0	7.9
	+		7.0	100	3.5	5.0
Positive Control	-	0.025	6.9	91	810	1200
1,2,3,4-Diepoxybutane	+	0.025	6.6	94	790	1200
Acetonitrile	+	5.0	7.0	100	3.0	4.3
	+	6.0	7.3	104	5.0	6.9
	+	7.0	7.6	109	3.0	4.0
	+	8.0	6.5	93	9.0	15
	+	9.0	2.5	36	27	108
	+	10.0	T*	T	T	T

---

*T = toxic.

Table 361

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
ACETONITRILE
Experiment 6

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml (x 10^{-7})	Percent	Per ml (x 10^{-3})	Per 10^5 Survivors
Negative Control						
DMSO	-		7.5	100	11	15
	+		7.8	100	6.5	8.4
Positive Control						
1,2,3,4-Diepoxybutane	-	0.025	8.3	111	1500	1800
	+	0.025	6.2	79	1100	1800
Acetonitrile	-	5.0	8.6	115	11	13
	-	6.0	7.9	105	5.0	6.3
	-	7.0	8.3	110	6.0	7.2
	-	8.0	7.8	104	13	17
	-	9.0	T[*]	T	T	T
	-	10.0	T	T	T	T
	+	5.0	7.8	100	8.0	10
	+	6.0	9.4	121	5.0	5.3
	+	7.0	8.1	104	9.0	11
	+	8.0	6.7	86	15	22
	+	9.0	6.8	87	30	44
	+	10.0	T	T	T	T

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[*] T = toxic.

Table 362

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
Acetonitrile
Experiment 7

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml (x 10^{-7})	Percent	Per ml (x 10^{-3})	Per 10^5 Survivors
Negative Control						
DMSO	-		8.2	100	7.5	9.2
	+		8.0	100	6.0	7.2
Positive Controls						
1,2,3,4-Diepoxybutane	-	0.025	6.6	80	1100	1700
	+	0.025	7.0	88	1100	1600
Acetonitrile	-	6.0	7.0	85	4.0	5.7
	-	7.0	6.1	74	2.0	3.3
	-	8.0	4.5	55	5.0	11
	-	9.0	5.6	68	14	25
	-	9.5	T[*]	T	T	T
	+	6.0	6.7	84	12	18
	+	7.0	6.5	81	5.0	7.7
	+	8.0	6.5	81	4.0	6.2
	+	9.0	T	T	T	T
	+	9.5	T	T	T	T

---

[*] T = toxic

Table 363

DIFFERENTIAL TOXICITY ASSAY
ACETONITRILE

Compound	Amount Added per Plate	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		E. coli				B. subtilis			
		W3110 (polA$^+$)		p3478 (polA$^-$)		H17 (rec$^+$)		M45 (rec$^-$)	
EXPERIMENT 1									
Negative control Kanamycin	0.1 mg	NT*		NT		10	10	10	10
Positive control Methylmethane sulfonate	1 mg	NT		NT		22	20	45	42
Acetonitrile	10 µl	NT		NT		no zone		no zone	
	20 µl	NT		NT		no zone		no zone	
EXPERIMENT 2									
Negative control Kanamycin	0.1 mg	10	10	10	10	15	16	16	17
Positive controls 1-phenyl-3,3-dimethyltriazene	1 mg	27	26	30	31				
Methylmethane sulfonate	1 mg					17	14	46	46
Acetonitrile	20 µl	no zone		no zone		no zone		no zone	
EXPERIMENT 3									
Negative control Kanamycin	0.1 mg	11	11	11	11	15	16	15	16
Positive control Methylmethane sulfonate	1 mg	15	14	25	23	17	17	50	47
Acetonitrile	20 µl	no zone		no zone		no zone		no zone	

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*NT, not tested.

TABLE 364

QUALITATIVE DIFFERENTIAL TOXICITY ASSAY

ACETONITRILE

Experiment 1

Compound	Amount of Compound Added per Plate	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		E. coli				B. subtilis			
		W3110 ($polA^+$)		p3478 ($polA^-$)		H17 (rec^+)		M45 (rec^-)	
Negative Controls									
Ampicillin	20 µg	21	20	20	21				
Chloramphenicol	20 µg					31	31	29	30
Positive Control									
Methylmethanesulfonate	1 mg	33	30	38	36	16	15	20	20
Acetonitrile	25 µℓ	7	7	7	7	8	8	8	8
	50	7	7	7	7	10	10	10	9

TABLE 365

QULITATIVE DIFFERENTIAL TOXICITY ASSAY

ACETONITRILE

Experiment

Compound	Amount of Compound Added per Plate	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		E. coli				B. subtilis			
		W3110 (polA$^+$)		p3478 (polA$^-$)		H17 (rec$^+$)		M45 (rec$^-$)	
Negative Control									
Chloramphenicol	20 µg	25	25	25	24	30	30	30	29
Positive Control									
Methylmethanesulfonate	1 mg	13	15	32	26	13	13	20	17
Acetonitrile	25 µℓ	7	7	7	7	8	7	7	8
	50	8	8	8	7	9	9	8	9

Chlorobenzene

S. typhimurium Plate Incorporation, E. coli WP-2 (Tables 366, 367). Chlorobenzene is flammable and hygroscopic; therefore, it was stored in a liquid nitrogen tank until used. The compound was tested twice in these assays on separate days, in duplicate, over a dose range of 0.01 to 5.0 μℓ/plate (~ 0.01 to 5.5 mg/plate; density ≈ 1.1). No reproducible dose-related increase in the number of revertants was observed. Toxicity, denoted by the appearance of pinpoint colonies and/or a thinning of the background lawn, was observed at 5.0 μℓ/plate in all cases with and without metabolic activation.

S. cerevisiae D3 (Tables 368, 369). Chlorobenzene was tested for recombinogenic activity over a concentration range of 0.0075 to 0.1% and retested over a narrower range of 0.02 to 0.06%. An increase in the number of recombinants was observed only in the presence of metabolic activation. This could be attributed to the ability of certain metabolites of chlorobenzene to cause mitotic recombination in the yeast. The increase observed in Experment 2 at 0.05% in the absence of metabolic activation was not reproducible. We conclude that chlorobenzene was negative without activation, positive with activation in this assay.

E. coli/B. subtilis Relative Toxicity (Table 370). This compound was equally toxic to recombination-proficient and -deficient strains of B. subtilis (strains H17 and M45, respectively) and to DNA polymerase-proficient and -deficient strains of E. coli (W3110 and p3478, respectively). Chlorobenzene apparently did not interact with and damage the bacterial DNA in this assay. Thus, this compound was negative in this test.

Table 366

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI WP2

Chlorobenzene
Experiment I

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate: TA1535		TA1537		TA1538		TA98		TA100		Tryptophan Revertants per Plate: WP2	
Negative control														
DMSO	-		24	24	7	5	13	14	27	31	100	84	29	44
	+		18	9	15	12	6	20	16	39	115	104	54	50
Positive controls														
Sodium azide	-	0.5 µg	310	244										
	-	1.0									404	453		
9-Aminoacridine	-	50.0			374	292								
2-Nitrofluorene	-	5.0					550	542	287	305				
AF2	-	0.1											1927	1988
2-Anthramine	-	1.0					18	15	24	27	92	112		
	+	1.0					330	354	274	292	409	347		
	-	2.5	18	27	11	17								
	+	2.5	154	158	102	90								
	-	10.0											44	55
	+	10.0											480	474
Chlorobenzene	-	0.01 µl	17	19	10	12	15	10	27	25	125	100	27	20
	-	0.05	17	16	8	15	14	13	20	20	97	92	30	35
	-	0.10	17	12	9	16	16	12	20	18	97	103	28	31
	-	0.50	21	19	6	15	13	10	19	18	108	97	39	29
	-	1.00	21	18	7	10	15	6	26	20	91	90	37	39
	-	5.00	T*	T	T	T	T	T	T	T	T	T	T	T
	+	0.01	13	9	16	11	19	25	37	30	96	102	55	36
	+	0.05	8	5	17	16	16	17	28	28	85	105	49	41
	+	0.10	12	8	15	14	19	13	30	27	100	100	51	48
	+	0.50	8	12	13	10	16	16	21	30	117	101	41	45
	+	1.00	5	7	12	8	19	20	31	30	C†	77	40	47
	+	5.00	T	T	7	11	16	19	30	25	81	86	T	T

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*T, toxic.

†C, contamination.

Table 367

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI WP2
Chlorobenzene
Experiment 2

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate TA1535		TA1537		TA1538		TA98		TA100		Tryptophan Revertants per Plate WP2	
Negative control														
DMSO	-		29	25	8	6	17	16	18	17	101	97	37	36
	+		10	10	12	13	15	20	32	29	85	92	43	36
Positive controls														
Sodium azide	-	0.5 µg	353	390										
	-	1.0									524	530		
9-Aminoacridine	-	50.0			635	385								
2-Nitrofluorene	-	5.0					1222	1161	576	618				
AF2	-	0.1											634	722
2-Anthramine	-	1.0					12	14	25	26	100	93		
	+	1.0					194	150	344	252	552	370		
	-	2.5	18	26	10	15								
	+	2.5	297	260	93	84								
	-	10.0											36	31
	+	10.0											296	218
Chlorobenzene	-	0.01 µl	25	18	7	8	9	9	18	23	85	78	38	29
	-	0.05	29	17	10	6	10	10	17	22	92	75	35	32
	-	0.10	31	17	6	5	13	9	15	15	100	72	43	33
	-	0.50	23	20	7	7	9	15	22	20	84	79	26	34
	-	1.00	20	15	4	6	13	8	14	19	77	65	35	36
	-	5.00	T	T*	T	T	T	T	T	T	T	T	T	T
	+	0.01	5	9	9	11	21	27	34	42	72	77	34	37
	+	0.05	9	11	12	12	19	25	28	36	66	68	37	45
	+	0.10	14	14	8	10	17	24	36	38	69	64	38	35
	+	0.50	7	14	9	14	21	25	31	41	54	66	36	48
	+	1.00	9	15	13	12	18	17	35	29	51	68	45	39
	+	5.00	T	T	T	T	T	T	T	T	T	T	T	T

---

*T, toxic.

Table 368

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

Chlorobenzene
Experiment 1

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors: Cells per ml (x 10^{-7})	Survivors: Percent	Mitotic Recombinants: Per ml (x 10^{-3})	Mitotic Recombinants: Per 10^5 Survivors
Negative control DMSO	-		6.3	100	3.5	5.6
	+		7.9	100	2.0	2.5
Positive control 1,2,3,4-Diepoxybutane	-	0.025	6.1	97	708	1200
	+	0.025	6.3	80	883	1400
Chlorobenzene	-	0.0075	6.6	105	3.0	4.6
	-	0.0100	8.9	141	3.0	3.4
	-	0.0250	4.3	68	2.0	4.7
	-	0.0500	4.9	78	3.0	6.1
	-	0.1000	T*		T	
	+	0.0075	7.2	91	1.0	1.4
	+	0.0100	6.8	86	4.0	5.9
	+	0.0250	4.6	58	6.0	13
	+	0.0500	4.0	51	9.0	23
	+	0.1000	T		T	

---

*T, toxic.

Table 369

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

Chlorobenzene
Experiment 2

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml (x 10^{-7})	Percent	Per ml (x 10^{-3})	Per 10^{5} Survivors
Negative control DMSO	-		5.8	100	7.0	12
	+		5.9	100	4.0	6.8
Positive control 1,2,3,4-Diepoxybutane	-	0.0025	7.4	128	620.0	840
	+	0.0025	3.5	59	343.0	980
Chlorobenzene	-	0.02	4.8	83	1.0	2.1
	-	0.03	6.1	105	2.0	3.3
	-	0.04	5.8	100	3.0	5.2
	-	0.05	3.7	64	8.0	22
	-	0.06	1.1	19	9.0	82
	+	0.02	4.7	80	4.0	8.5
	+	0.03	6.0	102	5.0	8.3
	+	0.04	4.2	71	3.0	7.1
	+	0.05	2.8	47	8.0	29
	+	0.06	0.4	7	7.0	180

Table 370

DIFFERENTIAL TOXICITY ASSAYS
Chlorobenzene

Compound	Amount	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		E. coli				B. subtilis			
		W3110 (polA$^+$)		p3478 (polA$^-$)		H17 (rec$^+$)		M45 (rec$^-$)	
EXPERIMENT 1									
Negative control Ampicillin	0.01 mg		18		16		11		11
Positive control Methylmethane sulfonate	1.0		30		39		9		49
Chlorobenzene	10.0 µl	10	10	10	10	10	10	10	10
	20.0	12	12	12	12	11	11	11	11
EXPERIMENT 2									
Negative control Ampicillin	0.01 mg	19	18	17	17	25	25	27	27
Positive control Methylmethane sulfonate	1.0	16	16	22	23	15	15	50	50
Chlorobenzene	10.0 µl	8	10	8	7	10	9	8	9
	20.0	11	11	11	11	11	11	11	11

Coal Tar (Flaked Pitch)

S. typhimurium Plate Incorporation (Tables 371, 372). The compound was first tested over a dose range of 10 to 5000 µg/plate without metabolic activation and 1 to 1000 µg/plate with activation. The sample was retested over a dose range of 10 to 5000 µg/plate without activation and 1 to 500 µg/plate with activation. A mutagenic response was obtained with strains TA1537, TA1538, TA98, and TA100 with metabolic activation; a weak, reproducible increase in the number of revertants was observed with TA100 without metabolic activation.

E. coli WP-2 (Tables 373, 374). The compound was tested twice over a dose range of 10 to 5000 µg/plate. Coal tar (flaked pitch) elicited a mutagenic repsonse only in the presence of metabolic activation. This response was both reproducible and dose-related.

S. cerevisiae D3 (Tables 375, 376). Coal tar (flaked pitch) was first tested over concentrations ranging from 0.1 to 2.5% and retested at 0.1 to 5.0%. No reproducible increase in the number of mitotic recombinants per 10^5 survivors was observed.

E. coli/B. subtilis Relative Toxicity (Tables 377, 378). Initially, 1.0 and 5.0 mg of the compound, dissolved in DMSO, were placed on a disc. Because the samples were inclined to precipitate and remain on the disc, lower doses of 0.5 and 1.0 mg were chosen for Experiment 2. At these doses, an extract was able to move off the disc and into the agar. Since all samples generally demonstrated equal zones of toxicity to both repair-proficient and repair-deficient strains of E. coli and B. subtilis, the results were negative.

Table 371

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

COAL TAR (FLAKED PITCH)

Experiment 1

Compound	Metabolic Activation	Amount of Compound Added/Plate	Histidine Revertants per Plate									
			TA1535		TA1537		TA1538		TA98		TA100	
Negative Control												
DMSO	-	50.0 µl	27	39	4	11	16	26	20	24	123	141
	+	50.0	24	18	36	27	37	52	56	55	134	127
Positive Controls												
Sodium Azide	-	1.0 µg	460	421							502	532
9-Aminoacridine	-	50.0			184	289						
2-Nitrofluorene	-	5.0					844	944	536	561		
2-Anthramine	-	1.0					13	12	31	26	108	115
	+	1.0					600	597	239	287	649	710
	-	2.5	28	32	13	15						
	+	2.5	220	263	175	178						
Coal Tar (Flaked Pitch)	-	10.0 µg	27	33	15	18	24	12	27	41	142	130
	-	50.0	36	36	17	12	20	26	38	39	150	148
	-	100.0	40	24	22	12	20	20	34	24	166	197
	-	500.0	26	40	29	17	15	20	38	43	217	211
	-	1,000.0	41	30	25	26	30	24	49	34	225	210
	-	5,000.0	38	23	28	27	32	50	43	45	197	191
	+	1.0 µg	13	20	31	36	32	29	42	49	140	154
	+	10.0	20	24	49	49	103	88	128	116	354	349
	+	50.0	16	19	114	123	354	347	354	347	737	719
	+	100.0	26	20	197	182	565	618	648	556	785	910
	+	500.0	20	20	298	280	1232	1228	1160	1251	1036	1129
	+	1,000.0	19	23	351	352	1417	1410	1336	1464	1030	1232

Table 372

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
COAL TAR (FLAKED PITCH)

Experiment 2

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate									
			TA1535		TA1537		TA1538		TA98		TA100	
Negative Control												
DMSO	-	50.0 µl	29	21	11	7	12	11	17	31	105	93
	+	50.0	10	16	15	23	33	39	51	44	104	113
Positive Controls												
Sodium Azide	-	1.0 µg	313	299							409	358
9-Aminoacridine	-	50.0			126	214						
2-Nitrofluorene	-	5.0					758	723	493	489		
2-Anthramine	-	1.0					19	12	36	38	129	92
	+	1.0					447	448	474	450	812	869
	-	2.5	19	26	12	14						
	+	2.5	215	190	182	235						
Coal Tar (Flaked Pitch)	-	10.0 µg	28	31	7	14	14	13	31	28	138	122
	-	50.0	38	16	7	14	15	18	30	31	142	114
	-	100.0	27	33	18	19	18	18	44	41	125	143
	-	500.0	35	20	18	20	19	15	32	45	174	152
	-	1,000.0	23	23	28	42	26	32	40	43	250	270
	-	5,000.0	16	15	24	31	23	29	44	41	164	157
	+	1.0	21	24	23	24	51	44	61	68	151	143
	+	5.0	20	18	28	31	48	48	101	91	212	189
	+	10.0	20	28	79	81	89	115	190	187	459	419
	+	50.0	29	30	128	90	136	140	212	233	529	491
	+	100.0	40	24	150	146	243	275	427	383	623	528
	+	500.0	26	13	220	186	392	347	515	466	649	640

Table 373

IN VITRO ASSAYS WITH ESCHERICHIA COLI WP2
COAL TAR (FLAKED PITCH)
Experiment 1

Compound	Metabolic Activation	Compound Added per Plate (µg)	Tryptophan Revertants per Plate	
Negative Control	-		20	23
DMSO	+	.	56	49
Positive Controls				
2-Anthramine	-	10.0	26	23
	+	10.0	175	186
AF-2	-	0.1	555	632
Coal Tar (Flaked Pitch)	-	10.0	14	26
	-	50.0	16	24
	-	100.0	19	24
	-	500.0	27	33
	-	1,000.0	12	30
	-	5,000.0	26	38
	+	10.0	25	49
	+	50.0	75	67
	+	100.0	124	119
	+	500.0	156	136
	+	1,000.0	189	152
	+	5,000.0	159	145

Table 374

IN VITRO ASSAYS WITH ESCHERICHIA COLI WP2
COAL TAR (FLAKED PITCH)
Experiment 2

Compound	Metabolic Activation	Compound Added per Plate (μg)	Tryptophan Revertants per Plate	
Negative Control				
DMSO	-		29	32
	+		43	32
Positive Controls				
2-Anthramine	-	10.0	39	48
	+	10.0	519	653
AF-2	-	0.1	223	219
Coal Tar (Flaked Pitch)	-	10.0	29	45
	-	50.0	40	40
	-	100.0	38	42
	-	500.0	44	56
	-	1,000.0	39	56
	-	5,000.0	50	50
	+	10.0	53	45
	+	50.0	103	139
	+	100.0	134	181
	+	500.0	170	203
	+	1,000.0	255	378
	+	5,000.0	185	279

Table 375

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

COAL TAR (FLAKED PITCH)

Experiment 1

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors: Cells per ml (x 10^{-7})	Survivors: Percent	Mitotic Recombinants: Per ml (x 10^{-3})	Mitotic Recombinants: Per 10^5 Survivors
Negative Control						
DMSO	-		7.0	100	6.0	8.6
	+		6.7	100	6.5	10
Positive Control 1,2,3,4-Diepoxybutane	-	0.025	4.0	57	750	1900
		0.025	3.1	46	800	1600
Coal Tar (Flaked Pitch)	-	0.1	6.5	93	11	17
	-	0.25	6.6	94	12	18
	-	0.5	5.7	81	6.0	11
	-	1.0	9.9	141	5.0	5.1
	-	2.5	7.1	101	8.0	11
	+	0.1	6.9	103	13	19
	+	0.25	6.3	94	8.0	13
	+	0.5	6.6	99	10	15
	+	1.0	6.6	99	13	20
	+	2.5	8.2	122	6.0	7.3

Table 376

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
COAL TAR (FLAKED PITCH)

Experiment 2

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml (x 10^{-7})	Percent	Per ml (x 10^{-3})	Per 10^5 Survivors
Negative Control						
DMSO	-		8.2	100	7.5	9.2
	+		8.0	100	6.0	7.2
Positive Control 1,2,3,4-Diepoxybutane	-	0.025	6.6	80	1100	1700
	+	0.025	7.0	88	1100	1600
Coal Tar (Flaked Pitch)	-	0.1	8.3	101	6.0	7.2
	-	0.25	7.9	96	1.0	1.3
	-	0.5	6.6	80	4.0	6.1
	-	1.0	7.6	93	2.0	2.6
	-	2.5	8.0	98	10	13
	-	5.0	8.4	102	7.0	8.3
	+	0.1	8.0	100	4.0	5.0
	+	0.25	7.4	93	8.0	11
	+	0.5	7.2	90	5.0	6.9
	+	1.0	7.6	95	12	16
	+	2.5	6.3	79	11	17
	+	5.0	7.0	88	10	14

Table 377

QUALITATIVE DIFFERENTIAL TOXICITY ASSAY

COAL TAR (FLAKED PITCH)

Experiment 1

Compound	Amount of Compound Added per Plate	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		E. coli				B. subtilis			
		W3110 ($polA^+$)		p3478 ($polA^-$)		H17 (rec^+)		M45 (rec^-)	
Negative Controls									
Ampicillin	20 μg	21	20	20	21				
Chloramphenicol	20					31	31	29	30
Positive Controls									
Methylmethanesulfonate	1 mg	33	30	38	36	16	15	20	20
Coal Tar (Flaked Pitch)	1 mg	10	9	10	10	10	11	10	11
	5	11	10	8	10	10	11	10	10

Table 378

QUALITATIVE DIFFERENTIAL TOXICITY ASSAY

COAL TAR (FLAKED PITCH)

Experiment 2

Compound	Amount of Compound Added per Plate	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		E. coli				B. subtilis			
		W3110 ($polA^+$)		p3478 ($polA^-$)		H17 (rec^+)		M45 (rec^-)	
Negative Control									
Chloramphenicol	20.0 µg	25	25	25	24	30	30	30	29
Positive Control									
Methylmethanesulfonate	1.0 mg	13	14	32	26	13	13	20	17
Coal Tar (Flaked Pitch)	0.5 mg	9	9	9	8	7	7	8	8
	1.0	9	9	9	10	8	8	8	8

Coal Tar (Bitumastic Component A)

S. typhimurium Plate Incorporation (Tables 379, 380). This material was first tested over a dose range of 5 to 1000 µg/plate with activation. This was followed by a second experiment in which the doses ranged from 1 to 500 µg/plate without activation and from 0.5 to 100 µg/plate with activation. A reproducible, dose-related increase in the number of revertants was obtained with strains TA1537, TA1538, TA98, and TA100 in the presence of metabolic activation, but only strain TA100 showed an increase in revertants without metabolic activation.

E. coli WP-2 (Tables 381, 382). This material was tested first over a dose range of 5 to 1000 µg/plate and then over a dose range of 10 to 5,000 µg/plate. A reproducible, dose-related increase in the number of revertants was observed in the presence of metabolic activation. No increase in revertants was demonstrated in the absence of metabolic activation.

S. cerevisiae D3 (Tables 383, 384). This material was tested at concentrations ranging from 0.05 to 5.0% and retested at 0.01 to 0.5%. A dose-related increase in the number of mitotic recombinants per 10^5 survivors was observed only in the absence of metabolic activation.

E. coli/B. subtilis Relative Toxicity (Tables 385, 386). Initially, 1.0 and 5.0 mg of the material, dissolved in DMSO, were placed on a disc. Because the samples were inclined to precipitate and remain on the disc, lower doses of 0.5 and 1.0 mg were chosen for Experiment 2. At these doses, an extract was able to move off the disc and into the agar. All samples generally demonstrated equal zones of toxicity to both repair-proficient and repair-deficient strains of E. coli and B. subtilis.

Table 379

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
COAL TAR (BITUMASTIC COMPONENT A)
Experiment 1

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate									
			TA1535		TA1537		TA1538		TA98		TA100	
Negative Control												
DMSO	-	50,0 µl	27	39	4	11	16	26	20	24	123	141
	+	50.0	24	18	36	27	37	52	56	55	134	127
Positive Controls												
Sodium Azide	-	1.0 µg	460	421							502	532
9-Aminoacridine	-	50.0			184	289						
2-Nitrofluorene	-	5.0					844	994	536	561		
2-Anthramine	-	1.0					13	12	31	26	108	115
	+	1.0					600	597	239	287	649	710
	-	2.5	28	32	13	15						
	+	2.5	220	263	175	178						
Bitumastic Component A	-	5.0 µg	24	29	18	14	19	20	30	32	129	130
	-	10.0	29	28	19	10	24	16	31	32	136	147
	-	50.0	39	33	15	18	16	17	36	37	137	166
	-	100.0	31	31	20	19	15	25	31	39	136	158
	-	500.0	53	30	21	24	29	28	36	50	202	172
	-	1,000.0	46	26	27	20	39	26	63	53	117	100
	+	1.0	27	21	38	36	40	39	69	66	146	136
	+	5.0	20	19	45	28	89	43	86	80	186	175
	+	10.0	26	29	55	40	80	99	113	102	261	245
	+	50.0	17	18	96	87	332	311	341	317	561	694
	+	100.0	26	26	123	136	545	561	541	542	701	758
	+	500.0	25	21	208	236	1013	1069	977	1152	985	825

Table 380

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
COAL TAR (BITUMASTIC COMPONENT A)
Experiment 2

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate									
			TA1535		TA1537		TA1538		TA98		TA100	
Negative Control												
DMSO	-	50.0 µl	29	21	11	7	12	11	17	31	105	93
	+	50.0	10	16	15	23	33	39	51	44	104	113
Positive Controls												
Sodium Azide	-	1.0 µg	313	299							409	358
9-Aminoacridine	-	50.0			126	214						
2-Nitrofluorene	-	5.0					758	723	493	489		
2-Anthramine	-	1.0					19	12	35	38	129	92
	+	1.0					447	448	474	450	812	869
	-	2.5	19	26	12	14						
	+	2.5	215	190	182	235						
Bitumastic Component A	-	1.0 µg	19	21	15	9	14	15	28	36	108	96
	-	5.0	18	24	9	8	20	12	21	20	101	99
	-	10.0	25	27	9	10	15	12	20	30	97	89
	-	50.0	33	21	12	10	13	18	27	40	134	109
	-	100.0	21	21	11	12	18	20	31	26	135	139
	-	500.0	19	20	23	19	30	22	31	39	188	173
	+	0.5 µg	12	16	16	16	44	39	61	53	130	123
	+	1.0	15	15	18	13	38	30	50	62	130	113
	+	5.0	15	22	16	19	30	30	60	54	140	130
	+	10.0	20	13	24	27	60	41	79	69	138	152
	+	50.0	19	12	55	68	172	162	200	198	382	370
	+	100.0	21	18	91	100	268	222	320	263	396	456

Table 381

IN VITRO ASSAYS WITH ESCHERICHIA COLI WP2

COAL TAR (BITUMASTIC COMPONENT A)

Experiment 1

Compound	Metabolic Activation	Compound Added per Plate (µg)	Tryptophan Revertants per Plate	
Negative Control	-		20	23
DMSO	+		56	49
Positive Controls				
2-Anthramine	-	10.0	26	23
	+	10.0	175	186
AF-2	-	0.1	555	632
Bitumastic Component A	-	5.0	25	24
	-	10.0	29	24
	-	50.0	22	33
	-	100.0	22	47
	-	500.0	24	26
	-	1,000.0	52	29
	+	5.0	27	40
	+	10.0	37	48
	+	50.0	46	50
	+	100.0	62	74
	+	500.0	86	96
	+	1,000.0	108	118

Table 382

IN VITRO ASSAYS WITH ESCHERICHIA COLI WP2

COAL TAR (BITUMASTIC COMPONENT A)

Experiment 2

Compound	Metabolic Activation	Compound Added per Plate (μg)	Tryptophan Revertants per Plate	
Negative Control	-		21	17
DMSO	+		25	28
Positive Controls				
2-Anthramine	-	10.0	17	16
	+	10.0	250	260
AF-2	-	0.1	183	165
Bitumastic Component A	-	10.0	17	17
	-	50.0	16	24
	-	100.0	18	21
	-	500.0	24	29
	-	1,000.0	26	31
	-	5,000.0	38	40
	+	10.0	26	26
	+	50.0	61	55
	+	100.0	52	63
	+	500.0	94	95
	+	1,000.0	90	97
	+	5,000.0	105	107

Table 383

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
COAL TAR (BITUMASTIC COMPONENT A)

Experiment 1

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($x\ 10^{-7}$)	Percent	Per ml ($x\ 10^{-3}$)	Per 10^5 Survivors
Negative Control						
DMSO	-		7.0	100	6.0	8.6
	+		6.7	100	6.5	10
Positive Control 1,2,3,4-Diepoxybutane	-	0.025	4.0	57	750	1900
	+	0.025	3.1	46	800	2600
Bitumastic Component A	-	0.05	6.3	90	8.0	13
	-	0.1	5.7	81	8.0	14
	-	0.5	3.8	54	18	47
	-	1.0	T*	T	T	T
	-	5.0	T	T	T	T
	+	0.05	6.0	90	11	18
	+	0.1	6.6	99	12	18
	+	0.5	3.0	45	6.0	20
	+	1.0	T		T	T
	+	5.0	T		T	T

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*T = toxic.

Table 384

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

COAL TAR (BITUMASTIC COMPONENT A)

Experiment 2

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($x 10^{-7}$)	Percent	Per ml ($x 10^{-3}$)	Per 10^5 Survivors
Negative Control						
DMSO	-		8.2	100	7.5	9.2
	+		8.0	100	6.0	7.2
Positive Control	-	0.025	6.6	80	1100	1700
1,2,3,4-Diepoxybutane	+	0.025	7.0	88	1100	1600
Bitumastic	-	0.01	10	122	10	10
Component A	-	0.05	8.3	101	4.0	4.8
	-	0.1	8.8	107	7.0	8.0
	-	0.25	8.2	100	8.0	9.8
	-	0.5	7.6	93	15	20
	+	0.01	7.3	91	11	15
	+	0.05	8.6	108	9.0	10
	+	0.1	9.5	119	11	12
	+	0.25	7.7	96	12	16
	+	0.5	6.4	80	11	17

Table 385

QUALITATIVE DIFFERENTIAL TOXICITY ASSAY

COAL TAR (BITUMASTIC COMPONENT A)

Experiment 1

Compound	Amount of Compound Added per Plate	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		E. coli				B. subtilis			
		W3110 (polA$^+$)		p3478 (polA$^-$)		H17 (rec$^+$)		M45 (rec$^-$)	
Negative Controls									
Ampicillin	**20 µg**	**21**	**20**	**20**	**21**				
Chloramphenicol	**20**					**31**	**31**	**29**	**30**
Positive Controls									
Methylmethanesulfonate	**1 mg**	**33**	**30**	**38**	**36**	**16**	**15**	**20**	**20**
Bitumastic, Component A	1 mg	9	10	9	9	11	11	8	10
	5	10	10	10	10	11	10	10	10

Table 386

QUALITATIVE DIFFERENTIAL TOXICITY ASSAY

COAL TAR (BITUMASTIC COMPONENT A)

Experiment 2

Compound	Amount of Compound Added per Plate	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		E. coli				B. subtilis			
		W3110 (polA+)		p3478 (polA−)		H17 (rec+)		M45 (rec−)	
Negative Control									
Chloramphenicol	20.0 µg	25	25	25	24	30	30	30	29
Positive Control									
Methylmethanesulfonate	1.0 mg	13	14	32	26	13	13	20	17
Bitumastic, Component A	0.5 mg	10	9	9	9	8	8	9	9
	1.0	9	10	9	9	7	8	8	8

Coal Tar (Bitumastic Component B)

S. typhimurium Plate Incorporation (Tables 387, 388). This material was first tested over a dose range of 1 to 1000 μg/plate and retested at 5 to 1000 μg/plate. A reproducible, dose-related increase in the number of revertants was observed with strains TA1535 and TA100, with and without activation. Metabolic activation enhanced the response with TA1535 but seemed to inhibit the full potential for mutation with TA100--i.e., at equal concentrations, the number of revertants was nearly doubled without activation.

E. coli WP-2 (Tables 389, 390). This material was tested first over a dose of 5 to 1000 μg/plate and then over a dose range of 10 to 5000 μg/plate. A mutagenic response, which was both reproducible and dose-related, was obtained only in the presence of metabolic activation. No mutagenic response was observed in the absence of metabolic activation.

S. cerevisiae D3 (Tables 391, 392). This material was first tested over concentrations ranging from 0.01 to 0.25% and retested at 0.001 to 0.1%. A reproducible, dose-related increase in the number of mitotic recombinants per 10^5 survivors was obtained only with metabolic activation. The increase in recombinants observed without activation was not considered to be dose-related.

E. coli/B. subtilis Relative Toxicity (Tables 393, 394). Initially, 1.0 and 5.0 mg of the material, dissolved in DMSO, were placed on a disc. Because the samples were inclined to precipitate and remain on the disc, lower doses of 0.5 and 1.0 mg were chosen for Experiment 2. At these doses, an extract was able to move off the disc and into the agar. All samples generally demonstrated equal zones of toxicity to both repair-proficient and repair-deficient strains of E. coli and B. subtilis.

Table 387

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
COAL TAR (BITUMASTIC COMPONENT B)
Experiment 1

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate									
			TA1535		TA1537		TA1538		TA98		TA100	
Negative Control												
DMSO	-	50,0 µl	27	39	4	11	16	26	20	24	123	141
	+	50.0	24	18	36	27	37	52	56	55	134	127
Positive Controls												
Sodium Azide	-	1.0 µg	460	421								
9-Aminoacridine	-	50.0									502	532
2-Nitrofluorene	-	5.0			184	289						
2-Anthramine	-	1.0					844	994	536	561		
	+	1.0					13	12	31	26		
	-	2.5	28	32	13	15	600	597	239	287	108	115
	+	2.5	220	263	175	178					649	710
Bitumastic Component B	-	1.0 µg	31	33	9	12	14	12	30	38	114	120
	-	10.0	36	30	14	10	17	19	32	20	173	173
	-	50.0	40	38	13	14	17	24	37	21	341	358
	-	100.0	41	43	19	8	9	10	27	31	418	458
	-	500.0	52	50	10	14	18	15	38	28	765	832
	-	1,000.0	57	44	14	12	12	13	38	31	883	893
	+	1.0 µg	14	19	27	36	21	50	38	51	92	90
	+	10.0	24	17	32	36	41	30	39	74	112	103
	+	50.0	43	57	30	37	43	30	56	53	195	191
	+	100.0	97	92	36	27	51	39	49	72	190	209
	+	500.0	235	207	38	30	50	52	55	76	429	431
	+	1,000.0	256	257	33	24	45	51	61	65	434	451

Table 388

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

COAL TAR (BITUMASTIC COMPONENT B)

Experiment 2

Compound	Metabolic Activation	Amount of Compound Added/Plate	Histidine Revertants per Plate									
			TA1535		TA1537		TA1538		TA98		TA100	
Negative Control												
DMSO	-	50.0 µl	29	21	11	7	12	11	17	31	105	93
	+	50.0	10	16	15	23	33	39	51	44	104	113
Positive Controls												
Sodium Azide	-	1.0 µg	313	299							409	358
9-Aminoacridine	-	50.0			126	214						
2-Nitrofluorene	-	5.0					758	723	493	489		
2-Anthramine	-	1.0					19	12	36	38	129	92
	+	1.0					447	448	474	450	812	869
	-	2.5	19	26	12	14						
	+	2.5	215	190	182	235						
Bitumastic Component B	-	5.0 µg	17	10	6	8	15	19	32	30	116	131
	-	10.0	18	16	7	5	12	20	36	28	165	150
	-	50.0	21	21	8	8	18	12	37	28	298	252
	-	100.0	32	32	4	6	15	19	31	36	346	354
	-	500.0	49	48	9	12	25	17	20	21	670	640
	-	1,000.0	50	39	6	7	12	14	29	31	675	614
	+	5.0	9	15	27	15	33	28	61	55	99	105
	+	10.0	12	19	18	14	37	39	61	49	103	117
	+	50.0	36	48	14	15	30	20	51	60	135	155
	+	100.0	103	101	14	16	32	21	50	64	158	206
	+	500.0	163	231	18	17	27	21	62	60	247	369
	+	1,000.0	357	300	31	18	28	20	66	76	432	436

Table 389

IN VITRO ASSAYS WITH ESCHERICHIA COLI WP2

COAL TAR (BITUMASTIC COMPONENT B)
Experiment 1

Compound	Metabolic Activation	Compound Added per Plate (µg)	Tryptophan Revertants per Plate	
Negative Control				
DMSO	-		20	23
	+		56	49
Positive Controls				
2-Anthramine	-	10.0	26	23
	+	10.0	175	186
AF-2	-	0.1	555	632
Bitumastic Component B	-	5.0	16	24
	-	10.0	18	33
	-	50.0	27	44
	-	100.0	27	26
	-	500.0	36	30
	-	1,000.0	33	31
	+	5.0	38	30
	+	10.0	22	39
	+	50.0	49	69
	+	100.0	60	58
	+	500.0	62	70
	+	1,000.0	82	81

Table 390

IN VITRO ASSAYS WITH ESCHERICHIA COLI WP2
COAL TAR (BITUMASTIC COMPONENT B)

Experiment 2

Compound	Metabolic Activation	Compound Added per Plate (µg)	Tryptophan Revertants per Plate	
Negative Control				
DMSO	-		29	32
	+		43	32
Positive Controls				
2-Anthramine	-	10.0	39	48
	+	10.0	519	653
AF-2	-	0.1	223	219
Bitumastic Component B	-	10.0	47	48
	-	50.0	57	45
	-	100.0	57	43
	-	500.0	57	47
	-	1,000.0	48	43
	-	5,000.0	46	47
	+	10.0	62	72
	+	50.0	58	111
	+	100.0	90	139
	+	500.0	145	153
	+	1,000.0	111	127
	+	5,000.0	60	60

Table 391

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
COAL TAR (BITUMASTIC COMPONENT B)

Experiment 1

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml (x 10^{-7})	Percent	Per ml (x 10^{-3})	Per 10^5 Survivors
Negative Control						
DMSO	-		8.2	100	7.5	9.2
	+		8.0	100	6.0	7.2
Positive Control 1,2,3,4-Diepoxybutane	-	0.025	6.6	80	1100	1700
	+	0.025	7.0	88	1100	1600
Bitumastic Component B	-	0.01	5.6	68	27	48
	-	0.025	6.7	82	47	70
	-	0.05	7.6	93	37	49
	-	0.1	5.8	71	35	60
	-	0.25	5.3	65	40	75
	+	0.01	7.6	95	4.0	5.3
	+	0.025	6.7	84	1.0	1.5
	+	0.05	4.4	55	20	45
	+	0.1	3.8	48	24	63
	+	0.25	3.1	39	43	140

Table 392

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
COAL TAR (BITUMASTIC COMPONENT B)

Experiment 2

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml (x 10^{-7})	Percent	Per ml (x 10^{-3})	Per 10^{5} Survivors
Negative Control						
DMSO	-		4.9	100	4.5	8.1
	+		3.8	100	5.0	15
Positive Control 1,2,3,4-Diepoxybutane	-	0.025	6.4	131	980	1,500
	+	0.025	7.3	192	1,000	1,400
Bitumastic Component B	-	0.001	7.5	153	8.0	11
	-	0.005	5.9	120	33	56
	-	0.01	6.3	129	30	48
	-	0.05	6.2	127	23	37
	-	0.1	5.9	120	19	32
	+	0.001	6.6	174	7.0	12
	+	0.005	7.6	200	5.0	6.6
	+	0.01	7.1	187	3.0	4.2
	+	0.05	6.2	163	35	56
	+	0.1	5.6	147	42	75

Table 393

QUALITATIVE DIFFERENTIAL TOXICITY ASSAY

COAL TAR (BITUMASTIC COMPONENT B)

Experiment 1

Compound	Amount of Compound Added per Plate	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		E. coli				B. subtilis			
		W3110 (polA$^+$)		p3478 (polA$^-$)		H17 (rec$^+$)		M45 (rec$^-$)	
Negative Controls									
Ampicillin	20 µg	21	20	20	21				
Chloramphenicol	20					31	31	29	30
Positive Controls									
Methylmethanesulfonate	1 mg	33	30	38	36	16	15	20	20
Bitumastic, Component B	1 mg	12	11	9	10	12	11	8	9
	5	10	10	10	10	12	12	9	9

Table 394

QUALITATIVE DIFFERENTIAL TOXICITY ASSAY

COAL TAR (BITUMASTIC COMPONENT B)

Experiment 2

Compound	Amount of Compound Added per Plate	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		E. coli				B. subtilis			
		W3110 (polA$^+$)		p3478 (polA$^-$)		H17 (rec$^+$)		M45 (rec$^-$)	
Negative Control									
Chloramphenicol	20.0 μg	25	25	25	24	30	30	30	29
Positive Control									
Methylmethanesulfonate	1.0 mg	13	14	32	26	13	13	20	17
Bitumastic, Component B	0.5 mg	9	10	9	9	9	10	8	8
	1.0	9	9	8	9	9	10	7	7

Coal Tar (Bitumastic Components A + B)

S. typhimurium Plate Incorporation (Tables 395, 396). This mixture was first tested over a dose range of 5 to 1000 µg/plate without metabolic activation and 1 to 500 µg/plate with metabolic activation; it was retested over a dose range of 5 to 1,000 µg/plate. The results for the combined mixture of components A and B (3.5:1) reflect the responses to the individual components in most cases. A reproducible, dose-related increase in the number of revertants was observed in strains TA1537, TA98 and TA100 without metabolic activation, and in all strains with metabolic activation.

E. coli WP-2 (Tables 397, 398). The mixture was tested first over a dose range of 5 to 1000 µg/plate and then over a dose range of 10 to 5000 µg/plate. A reproducible, dose-related increase in the number of revertants was observed in the presence of metabolic activation. Bitumastic Components A + B, when combined, did not appear to elicit a mutagenic response in the absence of metabolic activation.

S. cerevisiae D3 (Tables 399, 400). The mixture was first tested over concentrations ranging from 0.05 to 5.0% and then retested at 0.01 to 0.25%. An increase in the number of mitotic recombinants per 10^5 survivors was observed with and without metabolic activation.

E. coli/B. subtilis Relative Toxicity (Tables 401, 402). Initially, 1.0 and 5.0 mg of the mixture, dissolved in DMSO, were placed on a disc. Because the samples were inclined to precipitate and remain on the disc, lower doses of 0.5 and 1.0 mg were chosen for Experiment 2. At these doses, an extract was able to move off the disc and into the agar. All samples generally demonstrated equal zones of toxicity to both repair-proficient and repair-deficient strains of E. coli and B. subtilis. Thus, the mixture was negative in this test system.

Table 395

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
COAL TAR (BITUMASTIC COMPONENTS A + B)

Experiment 1

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate TA1535		TA1537		TA1538		TA98		TA100	
Negative Control												
DMSO	-	50.0 µl	27	39	4	11	16	26	20	24	123	141
	+	50.0	24	18	36	27	37	52	56	55	134	127
Positive Controls												
Sodium Azide	-	1.0 µg	460	421							502	532
9-Aminoacridine	-	50.0			184	289						
2-Nitrofluorene	-	5.0					844	994	536	561		
2-Anthramine	-	1.0					13	12	31	26	108	115
	+	1.0					600	597	239	287	649	710
	-	2.5	28	32	13	15						
	+	2.5	220	263	175	178						
Bitumastic Components A + B	-	5.0 µg	37	39	14	13	21	20	26	24	134	130
	-	10.0	45	33	18	13	26	14	25	31	152	135
	-	50.0	42	31	14	15	25	25	33	38	225	202
	-	100.0	38	32	20	25	21	14	33	24	274	245
	-	500.0	51	32	44	31	27	56	40	41	578	538
	-	1,000.0	45	34	39	39	39	27	62	78	880	946
	+	1.0	33	15	24	33	47	55	74	52	127	130
	+	5.0	15	25	40	33	50	48	69	57	166	135
	+	10.0	24	25	42	32	74	67	84	85	210	213
	+	50.0	19	20	91	84	183	178	218	213	494	451
	+	100.0	45	36	113	98	313	286	309	284	769	651
	+	500.0	65	49	197	135	631	564	635	667	855	822

Table 396

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

COAL TAR (BITUMASTIC COMPONENTS A + B)

Experiment 2

Compound	Metabolic Activation	of Compound Added per Plate	Histidine Revertants per Plate TA1535		TA1537		TA1538		TA98		TA100	
Negative Control												
DMSO	-	50.0 µl	29	21	11	7	12	11	17	31	105	93
	+	50.0	10	16	15	23	33	39	51	44	104	113
Positive Controls												
Sodium Azide	-	1.0 µg	313	299							409	358
9-Aminoacridine	-	50.0			126	214						
2-Nitrofluorene	-	5.0					758	723	493	489		
2-Anthramine	-	1.0					19	12	36	38	129	92
	+	1.0					447	448	474	450	812	869
	-	2.5	19	26	12	14						
	+	2.5	215	190	182	235						
Bitumastic Components A + B	-	5.0 µg	20	20	8	8	13	19	14	32	92	87
	-	10.0	25	22	13	8	16	16	27	24	92	105
	-	50.0	15	20	12	5	15	12	36	43	141	134
	-	100.0	18	32	12	15	20	24	37	34	198	208
	-	500.0	26	41	24	30	17	25	37	63	410	465
	-	1,000.0	36	37	39	22	30	40	80	86	565	635
	+	5.0 µg	9	12	13	19	36	30	41	56	99	112
	+	10.0	7	15	20	30	29	56	49	65	136	149
	+	50.0	14	14	27	29	53	65	78	90	161	186
	+	100.0	8	19	61	64	183	170	272	260	466	422
	+	500.0	29	12	103	104	341	280	378	374	548	570
	+	1,000.0	33	38	218	183	707	664	707	719	761	764

Table 397

IN VITRO ASSAYS WITH ESCHERICHIA COLI WP2
COAL TAR (BITUMASTIC COMPONENTS A + B)
Experiment 1

Compound	Metabolic Activation	Compound Added per Plate (μg)	Tryptophan Revertants per Plate	
Negative Control	-		20	23
DMSO	+		56	49
Positive Controls				
2-Anthramine	-	10.0	26	23
	+	10.0	175	186
AF-2	-	0.1	555	632
Bitumastic Components A + B	-	5.0	23	28
	-	10.0	21	20
	-	50.0	28	35
	-	100.0	29	32
	-	500.0	29	22
	-	1,000.0	43	42
	+	5.0	33	36
	+	10.0	40	47
	+	50.0	88	88
	+	100.0	117	102
	+	500.0	128	120
	+	1,000.0	137	139

Table 398

IN VITRO ASSAYS WITH ESCHERICHIA COLI WP_2
COAL TAR (BITUMASTIC COMPONENTS A + B)
Experiment 2

Compound	Metabolic Activation	Compound Added per Plate (µg)	Tryptophan Revertants per Plate	
Negative Control				
DMSO	–		29	32
	+		43	32
Positive Controls				
2-Anthramine	–	10.0	39	48
	+	10.0	519	653
AF-2	–	0.1	223	219
Bitumastic Components A and B	–	10.0	36	42
	–	50.0	33	36
	–	100.0	32	43
	–	500.0	38	53
	–	1,000.0	31	48
	–	5,000.0	30	47
	+	10.0	52	50
	+	50.0	74	62
	+	100.0	89	85
	+	500.0	92	98
	+	1,000.0	97	151
	+	5,000.0	108	99

Table 399

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
COAL TAR (BITUMASTIC COMPONENTS A + B)

Experiment 1

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors: Cells per ml ($x\ 10^{-7}$)	Survivors: Percent	Mitotic Recombinants: Per ml ($x\ 10^{-3}$)	Mitotic Recombinants: Per 10^5 Survivors
Negative Control						
DMSO	-		7.0	100	6.0	8.6
	+		6.7	100	6.5	10
Positive Control 1,2,3,4-Diepoxybutane	-	0.025	4.0	57	750	1900
	+	0.025	3.1	46	800	2600
Bitumastic Components A + B	-	0.05	4.8	69	47	98
	-	0.1	4.8	69	62	130
	-	0.5	2.1	30	78	370
	-	1.0	T*		T	T
	-	5.0	T		T	T
	+	0.05	5.7	89	8.0	14
	+	0.1	2.6	39	17	65
	+	0.5	T		T	T
	+	1.0	T		T	T
	+	5.0	T		T	T

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*T = toxic.

Table 400

IN VITRO ASSAYS WITH *SACCHAROMYCES CEREVISIAE* D3
COAL TAR (BITUMASTIC COMPONENTS A + B)
Experiment 2

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml (x 10^{-7})	Percent	Per ml (x 10^{-3})	Per 10^5 Survivors
Negative Control DMSO	-		8.2	100	7.5	9.2
	+		8.0	100	6.0	7.2
Positive Control 1,2,3,4-Diepoxybutane	-	0.025	6.6	80	1100	1700
	+	0.025	7.0	88	1100	1600
Bitumastic Components A + B	-	0.01	6.4	78	35	55
	-	0.025	6.4	78	34	53
	-	0.05	7.2	88	60	83
	-	0.1	6.7	84	68	100
	-	0.25	7.4	90	101	140
	+	0.01	7.1	89	8.0	11
	+	0.025	7.5	94	8.0	11
	+	0.05	7.9	99	15	19
	+	0.1	6.4	80	17	27
	+	0.25	3.8	48	53	140

Table 401

QUALITATIVE DIFFERENTIAL TOXICITY ASSAY

COAL TAR (BITUMASTIC COMPONENTS A + B)

Experiment 1

		Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		E. coli				B. subtilis			
Compound	**Amount of Compound Added per Plate**	W3110 (polA$^+$)		p3478 (polA$^-$)		H17 (rec$^+$)		M45 (rec$^-$)	
Negative Controls									
Ampicillin	20 μg	21	20	20	21				
Chloramphenicol	20					31	31	29	30
Positive Controls									
Methylmethanesulfonate	1 mg	33	30	38	36	16	15	20	20
Bitumastic, Components A + B	1 mg	9	8	8	8	9	10	10	10
	5	8	8	9	8	10	10	8	8

Table 402

QUALITATIVE DIFFERENTIAL TOXICITY ASSAY

COAL TAR (BITUMASTIC COMPONENTS A + B)

Experiment 2

Compound	Amount of Compound Added per Plate	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
		E. coli				B. subtilis			
		W3110 ($polA^+$)		p3478 ($polA^-$)		H17 (rec^+)		M45 (rec^-)	
Negative Control									
Chloramphenicol	20.0 μg	25	25	25	24	30	30	30	29
Positive Control									
Methylmethanesulfonate	1.0 mg	13	14	32	26	13	13	20	17
Bitumastic, Components A + B	0.5 mg	9	10	9	9	9	9	8	9
	1.0	9	9	9	9	8	8	8	8

Ethanol

S. typhimurium Plate Incorporation, E. coli WP-2 (Tables 403-406). Ethanol was tested once over a dose range of 0.5 to 100.0 µℓ/plate (~ 0.4 to 80 mg/plate) and then retested over a range of 10 to 200 µℓ/plate (~ 8.0 to 160 mg/plate). No reproducible dose-related increase in the number of revertants was observed in either of these experiments. Toxicity, denoted by the appearance of pinpoint colonies and/or a thinning of the background lawn, was observed in all strains at 200 µℓ/plate without metabolic activation. Doses less than 100 µℓ/plate were made by serial dilutions with H_2O and then 100 µℓ of the dilution was plated. For doses of 100 µℓ/plate and greater, the appropriate volume was applied directly to the plate.

Additional S. typhimurium testing was performed with ethanol in desiccators because of its volatility. No dose-related increase in the number of histidine-independent revertants was demonstrated. Preliminary experiments with ethanol had indicated that doses of 5 ml/desiccator and greater were toxic. Failure to reach toxicity at this concentration in Experiment 2 could be because more plates were in the desiccator. Ethanol was retested over a dose-range of 1 to 5 ml/desiccator. It did not appear to be mutagenic in either experiment.

S. cerevisiae D3 (Tables 407, 408). No recombinogenic activity was observed in this assay with ethanol. Ethanol was tested over a concentration range of 0.1 to 5.0% and was retested over a range of 1 to 5%. No toxic concentration was established. We conclude that ethanol was negative in this assay.

E. coli/B. subtilis Relative Toxicity (Table 409). The doses tested were 10 and 20 µℓ of undiluted chemical applied directly to the filter paper disc. Ethanol was not found to be differentially toxic to the repair-proficient or -deficient strains; however, it was not appreciably toxic to any of the four tester strains. This lack of toxicity may have resulted from evaporation or rapid diffusion of the test chemical through the aqueous-agar medium. We conclude that ethanol did not demonstrate genotoxic activity in this assay.

Table 403

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

ETHANOL

Experiment 1

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate TA1535	TA1537	TA1538	TA98	TA100	Tryptophan Revertants per Plate WP2
Negative control (H₂O)	-		24,32	8,14	15,17	33,17	99, 92	
	+		13,21	13,14	25,21	39, C*	88, 89	27,30 42,42
Positive controls								
Sodium azide	-	0.5 µg	353,390					
	-	1.0					524,530	
9-Aminoacridine	-	50		635,385				
2-Nitrofluorene	-	5.0			1222,1161	576,618		
AF2	-	0.1						634,722
2-Anthramine	-	1.0			12,14	25,26	100, 93	
	+	1.0			194,150	344,252	552,370	
	-	2.5	13,26	10,15				
	+	2.5	297,260	93,84				
	-	10						35,31
	+	10						296,218
Ethanol	-	0.5 µl	32,29	13, 6	16,14	27,24	100, 37	32,36
	-	1.0	32,29	8, 3	3,12	27,18	97,33	32,37
	-	5.0	25,16	10,12	3, 6	26,25	106, 35	40,38
	-	10.0	18,25	7, 9	15,10	30,20	33, 74	36,49
	-	50.0	18,20	3, 7	13,12	23,24	74, 76	37,31
	-	100.0†	20,25	4, 9	9, 5	13,13	66, 57	25,36
	+	0.5	14,18	14,15	18,24	31,30	105,111	43,44
	+	1.0	15,13	13,15	14,13	31,38	97, 99	47,47
	+	5.0	17,15	11.17	21,19	33,33	112,105	51,50
	+	10.0	25,15	17,17	26,16	30,37	116,107	48,53
	+	50.0	26,33	10,11	11,17	32,31	100,105	38,45
	+	100.0	32,32	9,14	20,19	26,39	97,103	27†,42

* C, contaminated.
† Pinpoint colonies.

Table 404

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

ETHANOL

Experiment 2

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate					Tryptophan Revertants per Plate
			TA1535	TA1537	TA1538	TA98	TA100	WP2
Negative control (H_2O)	-		15,17	9, 4	5,10	38,24	110,135	33,36
	+		9,16	11, 5	15,19	38,28	117,130	38,42
Positive controls								
Sodium azide	-	0.5 µg	126,162					
	-	1.0					368,370	
9-Aminoacridine	-	50		102,131				
2-Nitrofluorene	-	5.0			630,700	438,463		
AF2	-	0.1						476,597
2-Anthramine	-	1.0			18,17	40,29	147,102	
	+	1.0			486,572	545,423	724,667	
	-	2.5	9,12	8,10				
	+	2.5	226,210	201,206				
	-	10						21,25
	+							358,341
Ethanol	-	10 µl	15,22	11, 6	16, 9	21,23	113,125	35,32
	-	25	18,16	5, 3	10,17	23,23	106,120	43,30
	-	50	19,12	3, 2	6, 8	18,18	72, 81	32,32
	-	75	15,15	4, 4	7, 7	12,11	78, 80	32,49
	-	100	13,13	6, 5	5, 5	17,20	80, 68	34,30
	-	150	14*,10*	5, 5	8, 7	11,13	86, 87	29*,30*
	-	200	0, 2*	2*, 4*	5*, 6*	8*,15*	60*, 57*	28*,33*
	+	10	11,16	2, 6	15,15	32,37	132,137	40,40
	+	25	16,12	4, 6	16,23	33,41	121,137	39,40
	+	50	23,16	6, 6	15,17	27,28	99,101	36,40
	+	75	16,20	5, 4	19, 8	41,24	110,104	48,35
	+	100	13,18	5, 2	12,13	44,40	113,115	36,40
	+	150	14,10	3, 4	16, 7	24,31	98, 90	44,39
	+	200*	12, 8	2, 7	17, 7	22,15	31, 78	31,25

* Pinpoint colonies.

Table 405

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM IN DESICCATORS - EIGHT-HOUR EXPOSURE

ETHANOL

Experiment 1

Compound	Metabolic Activation	Amount of Compound in Desiccator	Average Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		15	11	18	21	145
	+		16	8	18	24	128
Positive control Epichlorohydrin	-	1.0 µl	368	10	21	18	423
	+	1.0	137	13	17	24	251
Ethanol	-	0.5 ml	19	5	18	26	155
	-	1.0	27	7	17	21	163
	-	2.0	15	8	20	21	138
	-	3.0	14	2	14	15	117
	-	4.0	10	4	19	15	98
	+	0.5	21	12	18	39	136
	+	1.0	23	7	16	37	128
	+	2.0	20	8	20	38	122
	+	3.0	15	7	20	27	122
	+	4.0	12	8	10	33	96

Table 406

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM IN DESICCATORS - EIGHT-HOUR EXPOSURE

ETHANOL

Experiment 2

Compound	Metabolic Activation	Amount of Compound in Desiccator	Average Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		27	8	15	64	153
	+		32	17	21	78	178
Positive control Epichlorohydrin	-	1 µl	635	10	15	55	722
	+	1	386	20	24	62	541
Ethanol	-	1 ml	29	6	9	63	149
	-	2	29	4	14	62	131
	-	3	24	4	9	64	150
	-	4	33	3	4	61	138
	-	5	21	3	7	40	134
	+	1	36	13	26	54	176
	+	2	24	7	30	68	158
	+	3	30	7	15	74	162
	+	4	18	9	9	65	140
	+	5	24	7	18	52	103

Table 407

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
ETHANOL

Experiment 1

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors Cells per ml (x 10^{-7})	 Percent	Mitotic Recombinants Per ml (x 10^{-3})	 Per 10^5 Survivors
Negative control (H_2O)	-		6.9	100	6.5	9.4
	+		7.8	100	3.5	4.5
Positive control 1,2,3,4-Diepoxybutane	-	0.025	7.5	109	583	780
	+	0.025	6.7	86	593	890
Ethanol	-	0.1	7.6	110	6.0	7.9
	-	0.5	7.0	101	5.0	7.1
	-	1.0	7.7	112	3.0	3.9
	-	5.0	7.7	112	6.0	7.8
	+	0.1	7.6	97	4.0	5.3
	+	0.5	6.0	77	6.0	10
	+	1.0	7.8	100	8.0	10
	+	5.0	8.2	105	7.0	8.5

Table 408

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
ETHANOL
Experiment 2

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors: Cells per ml (x 10^{-7})	Survivors: Percent	Mitotic Recombinants: Per ml (x 10^{-3})	Mitotic Recombinants: Per 10^5 Survivors
Negative control (H_2O)	-		6.5	100	3.0	4.6
	+		6.6	100	3.5	5.3
Positive control 1,2,3,4-Diepoxybutane	-	0.025	5.7	88	583	1000
	+	0.025	6.2	94	465	750
Ethanol	-	1	5.0	77	2.0	4.0
	-	2	5.1	78	3.0	5.9
	-	3	5.0	77	1.0	2.0
	-	4	6.4	98	5.0	7.8
	-	5	6.3	97	2.0	3.2
	+	1	3.4	52	2.0	5.9
	+	2	5.5	83	3.0	5.5
	+	3	6.1	92	5.0	8.2
	+	4	6.0	91	2.0	3.3
	+	5	5.9	89	3.0	5.1

Table 409

DIFFERENTIAL TOXICITY ASSAY

ETHANOL

Compound	Concentration	Zone of Inhibition of Growth (in mm, disc=6 mm)			
		E. coli		B. subtilis	
		W3110 (polA+)	p3478 (polA−)	H17 (rec+)	M45 (rec−)
EXPERIMENT 1					
Negative control Ampicillin	0.01 mg	20,20	18,17	25,26	28,27
Positive control Methylmethane sulfonate	1.0 mg	30,30	35,35	14,14	49,46
Ethanol	10 µl	6, 6	6, 6	6, 6	6, 6
	20	6, 6	6, 6	7, 7	7, 7
EXPERIMENT 2					
Negative control Ampicillin	0.01 mg	18	16	11	11
Positive control Methylmethane sulfonate	1.0 mg	30	39	9	49
Ethanol	10 µl	6, 6	6, 6	7, 6	7, 6
	20	7, 7	7, 7	8, 8	8, 9

Methanol

S. typhimurium Plate Incorporation, E. coli WP-2 (Tables 410-413). Methanol was tested once over a dose range of 0.5 to 100.0 µℓ/plate (~ 0.4 to 80 mg/plate). No reproducible dose-related increase in the number of revertants was observed in any of these experiments. No toxic response was obtained with methanol at 200 µℓ/plate. Doses less than 100 µℓ/plate were made by serial dilutions with H_2O, and then 100 µℓ of the dilution was plated. For doses of 100 µℓ/plate and greater, the appropriate volume was applied directly to the plate.

Additional S. typhimurium testing was performed with methanol in desiccators because of its volatility. No dose-related increase in the number of histidine-independent revertants was demonstrated. Methanol was tested once over a dose-range of 0.5 to 4 ml/desiccator and then was retested over a dose-range of 1 to 5 ml/desiccator. It did not appear to be mutagenic in either experiment.

S. cerevisiae D3 (Tables 414, 415). No recombinogenic activity was observed in this assay. Methanol was tested over concentrations ranging from 0.1 to 5.0% and was retested over a range of 1 to 5%.

E. coli/B. subtilis Relative Toxicity (Table 416). The doses tested were 10 and 20 µℓ of undiluted chemical applied directly to the filter paper disc. Methanol was not found to be differentially toxic to the repair-proficient or -deficient strains; however, it was not appreciably toxic to any of the four tester strains. This lack of toxicity may have resulted from evaporation or rapid diffusion of the test chemical through the aqueous-agar medium. We conclude that methanol did nct demonstrate genotoxic activity in this assay.

Table 410

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

METHANOL

Experiment 1

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate					Tryptophan Revertants per Plate
			TA1535	TA1537	TA1538	TA98	TA100	WP2
Negative control (H_2O)	-		24,32	8,14	15,17	33,17	99, 92	27,30
	+		13,21	13,14	25,21	39, C*	88, 99	42,42
Positive controls								
Sodium azide	-	0.5 µg	353,390					
	-	1.0					524,530	
9-Aminoacridine	-	50		635,385				
2-Nitrofluorene	-	5.0			1222,1161	576,618		
AF2	-	0.1						634,722
2-Anthramine	-	1.0			12,14	25,26	100, 93	
	+	1.5			194,150	344,252	552,370	
	-	2.5	13,26	10,15				
	+	2.5	297,260	93,84				
	-	10						35,31
	+	10						296,218
Methanol	-	0.5 µl	36,20	11,10	16,18	20,20	85, 88	25,37
	-	1.0	21,27	7,11	20,18	31,21	74, 93	33,44
	-	5.0	29,20	8,13	14,10	21,18	77, 91	45,39
	-	10.0	32,30	8, 7	12,12	26,24	80, 37	30,31
	-	50.0	27,30	5,12	7, 9	28,21	77, 72	27,26
	-	100.0	20,17	7, 7	14,12	25,16	63, 73	39,39
	+	0.5	14,17	17,19	24,25	31,32	101,105	48,37
	+	1.0	13, 9	11,17	15,26	31,32	38, 37	50,40
	+	5.0	8,14	17,13	19,15	30,32	96, 99	45,52
	+	10.0	17,18	17,12	19,23	29,33	37,112	49,41
	+	50.0	34,33	19,12	28,21	36, C	91,101	50,46
	+	100.0	17,24	19,12	20,20	31,30	34, 31	42,52

Table 411

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

METHANOL

Experiment 2

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate					Tryptophan Revertants per Plate
			TA1535	TA1537	TA1538	TA98	TA100	WP2
Negative control (H_2O)	-		15,17	9, 4	5,10	38,24	110,135	33,36
	+		9,16	11, 5	15,19	38,28	117,130	38,42
Positive controls								
Sodium azide	-	0.5 µg	126,162					
	-	1.0					368,370	
9-Aminoacridine	-	50		102,131				
2-Nitrofluorene	-	5.0			630,700	438,463		
AF2	-	0.1						476,597
2-Anthramine	-	1.0			18,17	40,29	147,102	
	+	1.0			486,572	545,423	724,667	
	-	2.5	9,12	8,10				
	+	2.5	226,210	201,206				
	-	10						21,25
	+							358,341
Methanol	-	10 µl	23,31	7, 7	20, 8	29,40	133,125	42,33
	-	25	13, C*	8, 6	11, 7	36,38	133,142	35,38
	-	50	28,31	12,11	16,15	32,43	126,143	37,42
	-	75	19,19	8, 5	17, 9	26,30	139,130	32,25
	-	100	30,25	8, 8	17,12	31,30	140,122	38,43
	-	200	39,34	10, 9	20,11	30,39	113,117	30,48
	+	10	31,23	10,11	27,19	48,37	138,138	43,32
	+	25	29,31	13,16	25,18	40,42	136,139	40,35
	+	50	31,34	19,10	22,24	47,40	147,131	35,37
	+	75	31,28	7,11	20,25	48,35	133,136	33,36
	+	100	30,36	18,13	26,20	40,43	141,135	38,42
	+	200	37,34	11,13	21,14	39,37	136,110	40,40

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* C, contaminated.

Table 412

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM IN DESICCATORS - EIGHT-HOUR EXPOSURE

METHANOL

Experiment 1

Compound	Metabolic Activation	Amount of Compound in Desiccator	Average Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		15	11	18	21	145
	+		16	8	18	24	128
Positive control Epichlorohydrin	-	1.0 µl	368	10	21	18	423
	+	1.0	137	13	17	24	251
Methanol	-	0.5 ml	24	12	18	21	135
	-	1.0	17	4	16	27	130
	-	2.0	26	7	8	19	110
	-	3.0	13	12	13	24	117
	-	4.0	27	6	12	18	116
	+	0.5	17	11	17	18	105
	+	1.0	21	13	15	29	117
	+	2.0	36	10	19	26	98
	+	3.0	25	11	32	35	101
	+	4.0	24	6	21	33	93

Table 413

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM IN DESICCATORS - EIGHT-HOUR EXPOSURE

METHANOL

Experiment 2

Compound	Metabolic Activation	Amount of Compound in Desiccator	Average Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		27	8	15	64	153
	+		32	17	21	78	178
Positive control	-	1 µl	635	10	15	55	722
Epichlorohydrin	+	1	386	20	24	62	541
Methanol	-	1 ml	28	12	11	53	170
	-	2	22	6	12	49	175
	-	3	23	5	19	45	166
	-	4	20	8	24	68	172
	-	5	24	14	15	66	155
	+	1	30	13	26	69	191
	+	2	36	20	21	79	198
	+	3	37	11	21	74	187
	+	4	27	8	25	67	180
	+	5	31	9	20	64	149

Table 414

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
METHANOL

Experiment 1

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml (x 10^{-7})	Percent	Per ml (x 10^{-3})	Per 10^5 Survivors
Negative control (H_2O)	-		6.9	100	6.5	9.4
	+		7.8	100	3.5	4.5
Positive control	-	0.025	7.5	109	583	780
1,2,3,4-Diepoxybutane	+	0.025	6.7	86	593	890
Methanol	-	0.1	8.3	120	2.0	2.4
	-	0.5	7.1	103	1.0	1.4
	-	1.0	4.6	67	3.0	6.5
	-	5.0	7.5	109	2.0	2.7
	+	0.1	6.5	83	2.0	3.1
	+	0.5	6.7	86	5.0	7.5
	+	1.0	6.6	85	3.0	4.6
	+	5.0	6.0	77	5.0	8.3

Table 415

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
METHANOL

Experiment 2

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml (x 10^{-7})	Percent	Per ml (x 10^{-3})	Per 10^5 Survivors
Negative control (H O)	-		6.5	100	3.0	4.6
	+		6.6	100	3.5	5.3
Positive control 1,2,3,4-Diepoxybutane	-	0.025	5.7	88	583	1023
	+	0.025	6.2	94	465	750
Methanol	-	1	8.1	125	1.0	1.2
	-	2	9.3	143	1.0	1.1
	-	3	7.8	120	2.0	2.6
	-	4	8.7	134	1.0	1.2
	-	5	7.2	111	2.0	2.8
	+	1	7.9	120	4.0	5.1
	+	2	8.0	121	4.0	5.0
	+	3	8.5	129	2.0	2.4
	+	4	7.2	109	1.0	1.4
	+	5	7.7	117	3.0	3.9

Table 416

DIFFERENTIAL TOXICITY ASSAY

METHANOL

Compound	Concentration	Zone of Inhibition of Growth (in mm, disc=6 mm)			
		E. coli		B. subtilis	
		W3110 (polA$^+$)	p3478 (polA$^-$)	H17 (rec$^+$)	M45 (rec$^-$)
EXPERIMENT 1					
Negative control Ampicillin	0.01 mg	20,20	18,17	25,26	28,27
Positive control Methylmethane sulfonate	1.0 mg	30,30	35,35	14,14	49,46
Methanol	10 20	6, 6 6, 6	6, 6 6, 6	6, 6 6, 6	6, 6 6, 6
EXPERIMENT 2					
Negative control Ampicillin	0.01 mg	18	16	11	11
Positive control Methylmethane sulfonate	1.0 mg	30	39	9	49
Methanol	10 20	6, 6 6, 6	6, 6 6, 6	6, 6 6, 6	6, 6 7, 6

Toluene

S. typhimurium Plate Incorporation, E. coli WP-2 (Tables 417, 418). Toluene was first tested over a dose range of 0.01 to 5.0 μℓ/plate (~ 0.0087 to 4.35 mg/plate; density ≈ 0.87) and then retested at higher levels ranging from 0.1 to 10.0 μℓ/plate (~ 0.087 to 8.7 mg/plate). Toxicity was observed with strains TA1535 and TA1538 at 10.0 μℓ/plate. No reproducible dose-related increase in the number of revertants was elicited by toluene in these assays.

S. cerevisiae D7 (Tables 419-421). Toluene did not induce mitotic crossing-over, mitotic gene conversion, or reverse mutation. The compound was tested from 0.005 to 0.5% in Experiment 1, from 0.001 to 0.01% in Experiment 2, and from 0.0025 to 0.025% in Experiment 3. Complete cell-killing was observed at 0.1% with and without metabolic activation.

E. coli, S. typhimurium Relative Toxicity (Tables 422-424). Several variations in the method of testing toluene in the differential toxicity assays were employed. Experiments 1 and 2 consisted of determining toxicity quantitatively, with and without preincubation; in Experiment 3, toxicity was determined by qualitative means. In Experiment 2, the decrease (compared to Experiment 1) in the overall toxic effect of the positive control compounds and of toluene to both pairs of strains may be due to the absence of preincubation. Toluene did not elicit differential toxicity and therefore did not cause DNA damage as detected in this assay.

SCE in CHO Cells (Tables 425-428). Toluene was dissolved in DMSO at a concentration of 0.5% for cytotoxicity testing with and without metabolic activation. At a concentration of 0.5%, toluene produced an oily film on the surface of the culture medium in the test without metabolic activation.

Table 426 presents the results of the cytotoxicity test of toluene in CHO cell cultures with metabolic activation. At a concentration of 0.5% toluene, the culture yielded M_1 metaphase cells. At 0.1% or less, M_2 metaphase cells were produced.

Table 427 presents the SCE frequencies in CHO cells exposed to toluene without metabolic activation. One sample exposed to 0.005% toluene could not be analyzed because chromosomes of the M_2 metaphase cells failed to harlequinize sufficiently. A one-way analysis of variance was conducted on the SCE frequencies of the other four concentrations of toluene and of the negative controls. The variance between treatment groups was not significantly greater than the variance within treatment groups.

Table 428 presents the SCE frequencies in CHO cells exposed to toluene with metabolic activation. At a concentration of 0.2% toluene, the cultures were not harvested because of excessive cytotoxicity. At a concentration of 0.1%, few or no metaphase cells were produced and those present were M_1. A one-way analysis of variance was conducted on SCE frequencies of the three lower concentrations of toluene and of the negative controls. The variance between treatment groups was not greater than the variance within treatment groups. However, the SCE frequencies observed by Cytogeneticist A for the negative control sample were unusually high, and a slight concentration-related increase in SCE frequencies was observed. Nonetheless, even when the negative control frequencies observed by Cytogeneticist B were used as the expected values in an analysis of variance (reducing the variance within samples to 0 for this pair of observations), the variance between samples was not significantly greater than the variance within samples.

For present purposes, we conclude that, under the conditions of our experiments, toluene did not induce SCEs in CHO cells, either with or without metabolic activation. In view of the somewhat high negative control values, it is desirable to repeat this assay in order to confirm this interpretation.

Micronucleus Test (Tables 429, 430). The dosing and sacrifice schedules are shown in Table 429. The incidences of micronuclei in PCEs and the PCE-to-RBC ratios are given in Table 430.

Toluene was negative at all dose levels and sampling times, with the β error ranging from 0.001 to 0.10 among the groups. The corn oil vehicle control groups were negative (β < 0.005-0.05) at all times. The TMP positive control group was positive (α < 0.001). The mean PCE-to-RBC ratios were within normal limits (0.50-1.50) for all toluene groups except for the low-dose group at 48 and 72 hours and the medium- and high-dose groups at 72 hours, which were slightly elevated. This rise indicates a slight increase in erythrocyte synthesis.

At 30 hours, benzene (route control) falls in the questionable range; at 48 hours it is positive. The numbers of micronuclei encountered were much lower than those previously reported (58) on oral administration of benzene. Whether this decreased effect is a result of the different route of administration used in this test or of other factors is unknown.

Under conditions used in our tests, toluene meets the criteria for a negative decision in the micronucleus test.

Table 417

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI
TOLUENE

Experiment 1

Compound	Metabolic Activation	Compound Added per Plate	Salmonella typhimurium										Escherichia coli	
			Histidine Revertants per Plate										Tryptophan Revertants	
			TA1535		TA1537		TA1538		TA98		TA100		WP2	
Negative Control														
DMSO	-		27	20	4	15	25	18	30	41	123	157	40	37
	+		15	12	21	24	26	18	48	48	115	116	39	46
Positive Control														
2-Anthramine	-	1.0 µg					12	13	39	36	161	142		
	+	1.0					502	518	404	464	966	947		
	-	2.5	36	28	16	14								
	+	2.5	470	426	235	347								
	-	10.0											36	44
	+	10.0											725	800
Sodium Azide	-	1.0	579	635							616	630		
9-Aminoacridine	-	50.0			263	245								
2-Nitrofluorene	-	5.0					796	813	360	604				
AF-2	-	0.1											625	646
Toluene	-	0.01 µl	28	26	7	13	17	10	38	43	122	129	40	55
	-	0.05	28	20	12	14	18	16	41	36	110	129	37	42
	-	0.1	23	28	13	10	12	13	37	44	100	137	49	43
	-	0.5	20	20	10	12	9	10	37	30	136	112	49	49
	-	1.0	28	15	8	15	12	17	42	20	104	123	46	50
	-	5.0	15	18	3	14	13	6	33	20	105	116	43	53
	+	0.01	19	20	13	18	21	16	54	45	138	108	49	49
	+	0.05	16	10	16	17	16	21	45	49	146	134	42	47
	+	0.1	10	14	10	25	22	24	52	49	130	129	52	56
	+	0.5	14	14	21	16	25	18	55	43	115	117	58	57
	+	1.0	14	11	14	15	31	25	48	32	116	125	51	57
	+	5.0	12	12	26	15	21	20	32	40	108	101	53	50

Table 418

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI
TOLUENE

Experiment 2

Compound	Metabolic Activation	Compound Added per Plate	Salmonella typhimurium										Escherichia coli	
			Histidine Revertants per Plate										Tryptophan Revertants	
			TA1535		TA1537		TA1538		TA98		TA100		WP2	
Negative Control														
DMSO	−		26	38	18	10	16	16	36	38	122	112		
	+		16	16	25	33	33	27	43	41	117	104	29	32
													43	32
Positive Controls														
2-Anthramine	−	1.0 µg					13	19	61	54	98	134		
	+	1.0					383	408	293	258	711	712		
	−	2.5	26	36	20	10								
	+	2.5	527	536	141	160								
	−	10.0											39	48
	+	10.0											519	653
Sodium Azide	−	1.0	719	700							699	726		
9-Aminoacridine	−	50.0			222	334								
2-Nitrofluorene	−	5.0					639	616	391	410				
AF-2	−	0.1											223	219
Toluene	−	0.1 µℓ	24	29	18	11	17	14	41	53	110	102	30	49
	−	0.5	27	27	17	7	16	15	39	44	114	108	30	48
	−	1.0	28	17	12	12	17	25	32	37	122	92	48	38
	−	5.0	15	21	10	12	15	13	27	19	84	96	37	49
	−	7.5	17	21	10	13	10	16	36	31	101	76	43	36
	−	10.0	30	PP*	13	12	PP	PP	25	20	79	88	30	48
	+	0.1	12	14	33	29	21	20	62	65	114	113	40	39
	+	0.5	11	19	21	20	36	29	65	45	104	104	42	40
	+	1.0	12	12	21	30	20	21	41	44	102	100	44	39
	+	5.0	18	10	29	37	25	25	40	42	89	104	45	36
	+	7.5	PP	8	26	28	24	27	48	38	90	97	56	57
	+	10.0	0	10	28	30	29	25	32	52	90	91	49	37

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*PP = pinpoint colonies.

Table 419

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D7
TOLUENE

Experiment 1

Compound	MA	% Conc.	Survivors: Cells Per ml ($\times 10^{-7}$)	Survivors: %	Crossing-Over: Mitotic Recombinants: Per ml ($\times 10^{-3}$)	Crossing-Over: Mitotic Recombinants: Per 10^5 Survivors	Crossing-Over: Total Aberrants: Per ml ($\times 10^{-3}$)	Crossing-Over: Total Aberrants: Per 10^5 Survivors	Gene Conversion: Convertants: Per ml ($\times 10^{-1}$)	Gene Conversion: Convertants: Per 10^6 Survivors	Reverse Mutation: Revertants: Per ml ($\times 10^{0}$)	Reverse Mutation: Revertants: Per 10^7 Survivors
Negative Control												
DMSO	-	10	5.6	100	1.3	2.3	9.4	17	52	9.3	13	2.3
	+	10	5.0	100	0.6	1.3	5.0	10	60	12	11	2.2
Positive Control												
1,2,3,4-Diepoxybutane	-	0.013	0.98	18	51	520	310	3200	1200	1200	47	48
	+	0.013	1.9	38	81	430	370	1900	1200	630	88	46
Toluene	-	0.005	6.2	110	3.8	6.1	10	16	17	2.7	0	0
	-	0.01	6.3	110	1.3	2.1	10	16	54	8.6	1.0	0.16
	-	0.05	6.9	120	1.3	1.9	8.8	13	53	7.7	3.0	0.43
	-	0.1	T*		T		T		T		T	
	-	0.5	T		T		T		T		T	
	+	0.005	5.3	110	2.5	4.7	10	19	12	2.3	1.0	0.19
	+	0.01	6.6	130	0	0	7.5	11	24	3.6	0	0
	+	0.05	6.9	140	0	0	16	23	24	3.5	0	0
	+	0.1	T		T		T		T		T	
	+	0.5	T		T		T		T		T	

Table 420

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D7

TOLUENE

Experiment 2

			Survivors		Crossing-Over				Gene Conversion		Reverse Mutation	
					Mitotic Recombinants		Total Aberrants		Convertants		Revertants	
Compound	MA	% Conc.	Cells Per ml ($\times 10^{-7}$)	%	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors	Per ml ($\times 10^{-1}$)	Per 10^6 Survivors	Per ml ($\times 10^{0}$)	Per 10^7 Survivors
Negative Control												
DMSO	-	10	4.4	100	2.5	5.7	12	27	70	16	19	4.3
	+	10	4.5	100	3.0	6.7	17	38	74	16	18	4.0
Positive Control												
1,2,3,4-Diepoxybutane	-	0.006	3.4	77	78	230	580	1700	1300	380	530	160
	+	0.006	3.8	84	120	320	720	1900	1400	370	490	130
Toluene	-	0.001	5.5	125	1.0	1.8	19	35	71	13	10	1.8
	-	0.0025	4.5	100	0	0	8.0	18	75	17	14	3.1
	-	0.005	4.3	98	0	0	13	30	67	16	8.0	1.9
	-	0.0075	5.0	114	3.0	6.0	22	44	85	17	11	2.2
	-	0.01	6.6	150	0	0	11	17	54	8.2	9.0	1.4
	+	0.001	4.3	96	0	0	18	42	82	19	10	2.3
	+	0.0025	3.7	82	2.0	5.4	13	35	47	13	9.0	2.4
	+	0.005	4.5	100	2.0	4.4	14	31	53	12	16	3.6
	+	0.0075	5.4	120	1.0	1.9	13	24	75	14	13	2.4
	+	0.01	4.7	104	0	0	15	32	88	19	14	3.0

Table 421

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D7

TOLUENE

Experiment 3

			Survivors		Crossing-Over				Gene Conversion		Reverse Mutation	
					Mitotic Recombinants		Total Aberrants		Convertants		Revertants	
Compound	MA	% Conc.	Cells Per ml ($\times 10^{-7}$)	%	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors	Per ml ($\times 10^{-1}$)	Per 10^6 Survivors	Per ml ($\times 10^0$)	Per 10^7 Survivors
Negative Control												
DMSO	-	10	3.8	100	2.0	5.3	7.5	20	80	21	19	5.0
	+	10	4.4	100	2.5	5.7	23	52	81	18	18	4.1
Positive Control												
1,2,3,4-Diepoxybutane	-	0.006	2.5	66	65	260	410	1600	800	320	230	92
	+	0.006	4.2	95	85	200	420	1000	1000	240	260	62
Toluene	-	0.0025	3.4	89	0	0	4.0	12	77	23	13	3.8
	-	0.005	3.9	100	0	0	11	28	82	21	2.0	0.51
	-	0.0075	3.4	89	2.0	5.9	6.0	18	82	24	5.0	1.5
	-	0.01	3.4	89	0	0	9.0	26	18	5.3	6.0	1.8
	-	0.025	0.62	16	0	0	3.8	61	13	21	1.0	1.6
	+	0.0025	3.9	89	1.3	3.3	11	28	73	19	8.0	2.1
	+	0.005	3.6	82	0	0	10	28	47	13	3.0	0.83
	+	0.0075	3.7	84	0	0	9.0	24	85	23	11	3.0
	+	0.01	3.5	80	0	0	10	29	22	6.3	3.0	0.86
	+	0.025	1.1	25	0	0	4.0	36	1.0	0.91	0	0

Table 422

QUANTITATIVE DIFFERENTIAL TOXICITY ASSAY

TOLUENE

Experiment 1

			E. coli							
			W3110 ($polA^+$)				p3478 ($pol\ A^-$)			
			Surviving Cells/ml ($\times 10^6$)			%	Surviving Cells/ml ($\times 10^6$)			%
Compound	Metabolic Activation	Amount of Compound per Plate	Plate 1	Plate 2	Average	Survival	Plate 1	Plate 2	Average	Survival
Negative Control										
DMSO	-	50.0 μℓ	181	187	184	100	163	168	165	100
	+	50.0	195	208	201	100	124	159	141	100
Positive Controls										
2-Anthramine	-	10.0 μg	175	191	183	99	116	159	138	84
	+	10.0	257	233	245	120	115	119	117	83
MMS	-	1.0 mg	158	194	176	96	0	0	0	0
Toluene	-	0.001 μℓ	48	86	67	36	52	78	65	39
	-	0.005	65	76	71	39	69	63	66	40
	-	0.010	C[*]	72	72	39	40	44	42	25
	+	0.001 μℓ	212	188	200	100	144	131	138	98
	+	0.005	116	C	116	58	98	79	89	63
	+	0.010	69	99	84	42	63	57	60	43

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[*] C = contaminated

Table 422 (Concluded)

			S. typhimurium							
			SL4525 (rfa) rec$^+$				SL4700(rfa) rec$^-$			
			Surviving			%	Surviving Cells/ml ($\times 10^6$)			%
Compound	Metabolic Activation	Amount of Compound per Plate	Plate 1	Plate 2	Average	Survival	Plate 1	Plate 2	Average	Survival
Negative Control										
DMSO	-	50.0 μℓ	459	413	436	100	256	257	257	100
	+	50.0	391	459	425	100	300	252	276	100
Positive Controls										
2-Anthramine	-	10.0 μg	453	402	428	98	293	317	305	119
	+	10.0	393	413	403	95	241	171	206	75
MMS	-	1.0 mg	233	289	261	60	0	0	0	0
Toluene	-	0.001 μℓ	254	223	239	55	143	155	149	58
	-	0.005	241	249	245	56	132	126	129	50
	-	0.010	140	145	143	33	125	89	107	42
	+	0.001 μℓ	255	249	252	89	171	269	220	80
	+	0.005	285	289	287	68	115	170	143	52
	+	0.010	C	229	229	54	C	101	101	37

---

[*] C = contaminated.

Table 423

QUANTITATIVE DIFFERENTIAL TOXICITY ASSAY

TOLUENE

Experiment 2

E. coli

Compound	Metabolic Activation	Amount of Compound per Plate	W3110 (polA$^+$) Surviving Cells/ml (×10^6) Plate 1	Plate 2	Average	% Survival	p3478 (pol A$^-$) Surviving Cells/ml (×10^6) Plate 1	Plate 2	Average	% Survival
Negative Control										
DMSO	-	50.0 µℓ	258	241	250	100	157	137	147	100
	+	50.0	290	279	285	100	184	209	197	100
Positive Controls										
2-Anthramine	-	20.0 µg	288	265	277	110	175	160	168	114
	+	20.0	259	281	276	95	187	179	183	93
MMS	-	1.0 mg	249	200	225	90	104	95	100	68
Toluene	-	0.001 µℓ	240	240	240	96	163	162	163	110
	-	0.005	237	221	229	92	141	152	147	100
	-	0.010	223	215	219	88	129	147	138	94
	+	0.001	266	242	254	89	145	170	158	80
	+	0.005	235	222	229	80	132	144	138	70
	+	0.010	229	220	225	79	135	156	146	74

Table 423 (Concluded)

			S. typhimurium							
			SL4525 (rfa) rec$^+$				SL4700(rfa) rec$^-$			
			Surviving Cell/ml $(\times 10^6)$			%	Surviving Cells/ml $(\times 10^6)$			%
Compound	Metabolic Activation	Amount of Compound per Plate	Plate 1	Plate 2	Average	Survival	Plate 1	Plate 2	Average	Survival
Negative Control										
DMSO	−	50.0 μℓ	846	725	786	100	627	787	707	100
	+	50.0	854	796	825	100	664	703	684	100
Positive Controls										
2-Anthramine	−	20.0 μg	767	792	780	99	660	652	656	93
	+	20.0	848	821	835	101	320	373	347	51
MMS	−	1.0 mg	663	633	648	82	0	0	0	0
Toluene	−	0.001 μℓ	773	842	808	103	657	680	669	95
	−	0.005	781	775	778	99	604	761	683	97
	−	0.010	667	689	678	86	619	689	654	93
	+	0.001	867	845	857	104	764	C	764	112
	+	0.005	C*	843	843	102	753	700	727	106
	+	0.010	769	751	760	92	685	729	707	103

---

*C = contaminated.

Table 424

QUALITATIVE DIFFERENTIAL TOXICITY ASSAY

TOLUENE

Experiment 3

Compound	Metabolic Activation	Amount of Compound per Plate	Zone of Inhibition of Growth (in mm, disc = 6 mm)							
			E. coli				S. typhimurium			
			W3110 (polA$^+$)		p3478 (polA$^-$)		SL4525(rfa) (rec$^+$)		SL4500(rfa) (rec$^-$)	
Negative Control										
Ampicillin	-	20.0 µg	20	21	18	17	20	20	21	19
	+	20.0	18	20	18	16	19	19	20	20
Chloramphenicol	-	10.0 µg	19	18	15	14	21	20	22	25
	+	10.0	15	17	18	20	23	20	25	24
Positive Control										
MMS	-	0.4 mg	7	7	14	13	7	7	34	32
Toluene	-	10.0 µℓ	8	8	8	8	9	9	8	8
	-	20.0	8	8	8	8	8	8	8	8
	+	10.0	10	10	9	9	10	8	9	9
	+	20.0	10	11	10	10	9	9	9	9

Table 425

CYTOTOXICITY TEST OF CHINESE HAMSTER OVARY CELLS EXPOSED TO TOLUENE FOR 21.5 HOURS WITHOUT METABOLIC ACTIVATION

Treatment	Number of Divisions in Bromodeoxyuridine (BrdU)[*]
Negative control (1% DMSO)	M_2
Toluene	
0.0008%	
0.004%	M_2
0.02%	M_2
0.1%	M_2
0.5%	---[†]
	---[†]
Positive control (10^{-3} M ethyl methanesulfonate)	M_2

---

[*] M_2, two divisions in BrdU.

[†] Not harvested due to excessive cytotoxicity.

Table 426

CYTOTOXICITY TEST OF CHINESE HAMSTER OVARY CELLS EXPOSED TO TOLUENE FOR 2 HOURS WITH METABOLIC ACTIVATION

Treatment	Number of Divisions in Bromodeoxyuridine (BrdU)[*]
Negative control (1% DMSO)	M_2
Toluene	
0.0008%	---[†]
0.004%	M_2
0.02%	M_2
0.1%	$M_2 > M_1$[‡]
0.5%	M_1
Positive control (10^{-3} M dimethylnitrosamine)	M_2

---

[*] M_1, one division in BrdU; M_2, two divisions in BrdU.

[†] This sample was lost because of experimental error.

[‡] The cell type to the left of the > sign was in excess.

Table 427

SISTER CHROMATID EXCHANGE (SCE) FREQUENCIES IN CHINESE HAMSTER OVARY (CHO) CELLS EXPOSED TO TOLUENE FOR 21.5 HOURS WITHOUT METABOLIC ACTIVATION

Treatment	Cytogeneticist[*]	No. of SCEs	No. of Chromosomes	Chromosomes/Cell (mean ± SD)	SCEs/Cell[†] (mean ± SE)	SCEs/ Chromosome[†] (mean ± SE)
Negative control (1% DMSO)	A	252	474	18.96 ± 0.84	10.08 ± 0.63	0.532 ± 0.032
	B	300	478	19.12 ± 0.73	12.00 ± 0.69	0.628 ± 0.036
Toluene						
0.0025%	A	252	479	19.16 ± 0.47	10.08 ± 0.64	0.526 ± 0.033
	B	225	480	19.20 ± 0.58	9.00 ± 0.60	0.469 ± 0.031
0.005%	A	245	483	19.32 ± 0.63	9.80 ± 0.63	0.507 ± 0.032
	---[‡]	---	---	---	---	---
0.01%	A	250	496	19.84 ± 0.55	10.00 ± 0.63	0.504 ± 0.032
	B	199	488	19.52 ± 0.82	7.96 ± 0.56	0.408 ± 0.029
0.02%	A	260	475	19.00 ± 0.50	10.40 ± 0.64	0.547 ± 0.034
	B	262	485	19.40 ± 0.91	10.48 ± 0.65	0.540 ± 0.033
0.04%	A	302	469	18.76 ± 0.83	12.08 ± 0.70	0.644 ± 0.037
	B	258	482	19.28 ± 0.74	10.32 ± 0.64	0.535 ± 0.033
Positive control (10^{-3} M ethyl methanesulfonate)	A	880	477	19.08 ± 0.76	35.20 ± 1.19	1.845 ± 0.062
	B	1049	477	19.08 ± 0.86	41.96 ± 1.30	2.199 ± 0.068

---

[*] Each cytogeneticist analyzed 25 cells per sample.

[†] A one-way analysis of variance comparing the SCE frequencies in CHO cells exposed to toluene at 0.0025%, 0.01%, 0.02%, and 0.04%, and to the negative control indicated that the variance between treatment groups was not significantly greater than the variance within treatment groups.

[‡] This sample was not analyzed because of poor harlequinization of 2nd division chromosomes.

Table 428

SISTER CHROMATID EXCHANGE (SCE) FREQUENCIES IN CHINESE HAMSTER OVARY (CHO) CELLS EXPOSED TO TOLUENE FOR 2 HOURS WITH METABOLIC ACTIVATION

Treatment	Cytogeneticist[*]	No. of SCEs	No. of Chromosomes	Chromosomes/Cell (mean ± SD)	SCEs/Cell[†] (mean ± SE)	SCEs/Chromosome[†] (mean ± SE)
Negative control (1% DMSO)	A	629	484	19.36 ± 0.95	25.16 ± 1.00	1.300 ± 0.052
	B	360	480	19.20 ± 0.65	14.40 ± 0.76	0.750 ± 0.040
Toluene						
0.0125%	A	367	480	19.20 ± 1.15	14.68 ± 0.77	0.765 ± 0.040
	B	372	475	19.00 ± 0.71	14.88 ± 0.77	0.783 ± 0.041
0.025%	A	414	481	19.24 ± 1.09	16.56 ± 0.81	0.861 ± 0.042
	B	486	468	18.72 ± 0.84	19.44 ± 0.88	1.038 ± 0.047
0.05%	A	429	459	18.36 ± 1.70	17.16 ± 0.83	0.935 ± 0.045
	B	452	475	19.00 ± 0.65	18.08 ± 0.85	0.952 ± 0.045
0.1%	---[‡]	---	---	---	---	---
0.2%	---[§]	---	---	---	---	---
Positive control (10^{-3} M dimethylnitrosamine)	A	782	480	19.20 ± 0.82	31.28 ± 1.12	1.629 ± 0.058
	B	1045	474	18.96 ± 0.84	41.80 ± 1.29	2.205 ± 0.068

---

[*] Each cytogeneticist analyzed 25 cells per sample.

[†] A one-way analysis of variance comparing the SCE frequencies in CHO cells exposed to toluene and to the negative control indicated that the variance between treatment groups was not greater than the variance within treatment groups.

[‡] Insufficient M_2 metaphase cells for analysis.

[§] Cultures were not harvested because of excessive cytotoxicity.

Table 429

DOSING AND SACRIFICE SCHEDULE

Compound	No. of Animals	Dose at 0 and 24 Hr	Mean Body Wt. Change (g)	Number Sacrificed 30 hr	48 hr	72 hr	No. of Fatalities
Corn oil - Vehicle control	24	10 ml/kg	+0.5	8	8	8	0
TMP in 5 ml/kg DMSO - Positive control	8	1.25 g/kg	-0.9	8	-	-	0
Benzene in 10 ml/kg corn oil Route control	12	0.25 ml/kg	-1.4	8	4	-	0
Toluene in 10 ml/kg corn oil							
Low dose	32	250 mg/kg	+0.8	8	8	8	0
Medium dose	32	500 mg/kg	+0.3	8	8	8	0
High dose	32	1000 mg/kg	-0.75	8	8	8	0

Table 430

INCIDENCE OF MICRONUCLEATED POLYCHROMATOPHILIC ERYTHROCYTES*

(500 PCE scored per animal)

Group	30 Hours			48 Hours			72 Hours		
	Animal No.	PCE with MN	PCE/RBC	Animal No.	PCE with MN	PCE/RBC	Animal No.	PCE with MN	PCE/RBC
Corn oil Vehicle control 2 x 10 ml/kg	C0	0	1.7	A0	1	1.8	B0	1	1.3
	C1	1	1.5	A1	1	1.8	B1	2	1.5
	C2	2	1.6	A2	0	1.2	B2	2	1.2
	C3	2	1.2	A3	2	2.2	B3	2	1.0
	E0	2	0.6	D0	2	1.2	F0	1	1.5
	E1	1	0.7	D1	0	0.7	F1	1	2.0
	E2	0	0.5	D2	0	1.4	F2	0	1.3
	E3	1	0.6	D3	1	1.0	F3	1	0.8
Total		9			7			10	
Mean			1.1			1.4			1.3
TMP Positive control 2 x 1.25 g/kg in 10 ml/kg DMSO	10o	59							
	10b	44							
	11o	7							
	11r	12							
	11ℓ	20							
	11b	14							
	12o	23							
	12r	19							
Total		198							
Mean									
Benzene Route control 2 x 0.25 ml/kg	G0	4	1.6	I0	2	1.4			
	G1	4	0.7	I1	1	1.1			
	G2	4	0.7	I2	4	0.8			
	G3	0	1.2	I3	4	0.6			
	H0	0	1.1						
	H1	4	1.7						
	H2	1	1.0						
	H3	0	1.3						
Total		13			11				
Mean			1.2			1.0			

(Continued)

Table 430 (Concluded)

	30 Hours			48 Hours			72 Hours		
Group	Animal No.	PCE with MN	PCE/RBC	Animal No.	PCE with MN	PCE/RBC	Animal No.	PCE with MN	PCE/RBC
Toluene 2 x 250 mg/kg	K0	1	1.3	L0	2	1.2	M0	0	1.8
	K1	1	1.3	L1	2	0.9	M1	1	1.2
	K2	1	0.8	L2	0	1.2	M2	1	2.4
	K3	0	1.6	L3	1	1.7	M3	0	1.0
	N0	2	1.2	P0	0	1.1	Q0	1	1.5
	N1	0	1.0	P1	1	1.8	Q1	0	1.3
	N2	1	0.5	P2	1	2.3	Q2	1	2.6
	N3	1	0.6	P3	1	2.7	Q3	0	1.0
Total		7			8			4	
Mean			1.0			1.6			1.6
Toluene 2 x 500 mg/kg	R0	0	2.5	T0	0	0.8	S0	1	1.6
	R1	0	1.0	T1	0	1.2	S1	1	1.0
	R2	1	2.2	T2	0	0.6	S2	1	2.0
	R3	1	1.6	T3	1	0.8	S3	1	2.9
	V0	0	0.8	Y0	0	2.3	W0	0	1.7
	V1	0	0.7	Y1	1	1.2	W1	0	1.8
	V2	2	0.7	Y2	0	1.6	W2	1	0.8
	V3	2	1.3	Y3	1	1.2	W3	1	1.8
Total		6			5			6	
Mean			1.4			1.2			1.7
Toluene 2 x 1000 mg/kg	CC0	2	1.0	BB0	1	1.1	AA0	1	1.1
	CC1	0	1.0	BB1	0	1.0	AA1	0	1.1
	CC2	1	1.2	BB2	1	0.8	AA2	1	0.9
	CC3	0	0.7	BB3	3	1.1	AA3	2	1.3
	EE0	1	0.6	dd0	0	1.2	FF0	1	3.3
	EE1	0	1.3	DD1	2	1.4	FF1	2	2.8
	EE2	1	1.2	DD2	1	2.0	FF2	0	1.6
	EE3	1	1.2	DD3	3	1.9	FF3	1	2.0
Total		6			11			8	
Mean			1.0			1.3			1.8

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* PCE = polychromatophilic erythrocytes, RBC = mature erythrocytes, MN = micronucleus.

Vinyl Fluoride

S. typhimurium Plate Incorporation (Tables 431-433). Vinyl fluoride gas was tested in desiccators over a wide range of concentrations--from 1.0% to 25%. The absence of data for strain TA1538 in Experiment 1 is due to contamination of the culture on that day. Since the increase in revertants observed in Experiments 2 and 3 for strain TA100 is not a two-fold increase and is not dose-related, we do not interpret the data as indicating a true mutagenic effect. We conclude that vinyl fluoride is not mutagenic in this test system.

E. coli WP-2 (Tables 434, 435). Vinyl fluoride was tested in desiccators over a wide range of concentrations from 1 to 25% in the first experiment and 5 to 25% in the second experiment, with three plates per concentration. The increase in tryptophan revertants observed in Experiment 1 was neither reproducible nor dose-related. Therefore, these data do not indicate a true mutagenic effect. We conclude that vinyl fluoride is not mutagenic in this system.

S. cerevisiae D3 (Tables 436, 437). The yeast was exposed to vinyl fluoride in desiccators for 48 hours. The positive control chemical was 1,2,3,4-diepoxybutane, a mutagen known to cause recombination in the yeast organism. Vinyl fluoride was first tested over a concentration range of 1 to 25%. The compound was retested over ranges of 5 to 25%. Some killing was observed at the highest concentration (25%). No reproducible dose-related increase in the number of mitotic recombinants was observed in either experiment. We conclude that vinyl fluoride does not induce mitotic recombination in the S. cerevisiae D3 assay, either in the presence or absence of a rat liver metabolic activation system.

Table 431

IN VITRO ASSAYS WITH *SALMONELLA TYPHIMURIUM* IN DESICCATORS - EIGHT-HOUR EXPOSURE
VINYL FLUORIDE

Experiment 1

Compound	Metabolic Activation	Percent of Compound in Desiccator	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative Control:	-		32	9	C*	21	122
	+		31	18	C	41	132
Positive Control: Ethylene oxide	-	0.06	162	15	C	20	629
	+	0.06	252	16	C	43	837
Vinyl fluoride	-	1.0	18	4	C	26	117
	-	5.0	35	6	C	21	141
	-	10.0	28	5	C	15	95
	-	25.0	31	2	C	25	123
	+	1.0	16	14	C	42	109
	+	5.0	8	11	C	41	104
	+	10.0	9	12	C	46	105
	+	25.0	13	12	C	35	129

---

*C, contaminated.

Table 432

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM IN DESICCATORS - EIGHT-HOUR EXPOSURE
VINYL FLUORIDE

Experiment 2

Compound	Metabolic Activation	Percent of Compound in Desiccator	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative Control:	-		34	15	20	37	155
	+		24	16	20	41	162
Positive Control: Ethylene oxide	-	0.06	325	12	18	53	497
	+	0.06	566	30	41	41	542
Vinyl fluoride	-	1.0	25	7	19	24	161
	-	2.5	31	13	19	38	161
	-	5.0	C*	12	19	38	182
	-	10.0	39	14	19	42	171
	-	25.0	35	8	8	27	174
	+	1.0	27	21	24	32	173
	+	2.5	30	20	30	49	164
	+	5.0	25	20	32	32	218
	+	10.0	30	22	32	51	141
	+	25.0	32	21	26	54	162

---

*C, contaminated.

Table 433

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM IN DESICCATORS - EIGHT-HOUR EXPOSURE VINYL FLUORIDE

Experiment 3

Compound	Metabolic Activation	Percent of Compound in Desiccator	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative Control:	-		36	10	16	38	123
	+		16	16	33	52	136
Positive Control: Ethylene oxide	-	0.06	1187	30	27	56	1101
	+	0.06	1566	38	28	74	1214
Vinyl fluoride	-	1.0	43	14	18	39	142
	-	2.5	44	8	19	36	135
	-	5.0	68	11	12	33	207
	-	10.0	38	20	17	38	129
	-	25.0	62	17	24	37	159
	+	1.0	25	26	23	44	149
	+	2.5	18	19	21	51	140
	+	5.0	60	31	26	52	188
	+	10.0	33	30	26	54	147
	+	25.0	72	24	27	52	148

Table 434

IN VITRO ASSAYS WITH *ESCHERICHIA COLI* WP2 IN DESICCATORS - EIGHT-HOUR EXPOSURE

VINYL FLUORIDE

Experiment 1

Compound	Metabolic Activation	Percent of Compound in Desiccator	Tryptophan Revertants per Plate		
Negative Control	-		17	24	20
	+		31	23	26
Positive Control					
Ethylene oxide	-	0.06	591	537	465
	+	0.06	570	536	594
Vinyl fluoride	-	1.0	81	54	64
	-	5.0	57	52	37
	-	10.0	38	44	31
	-	25.0	42	31	41
	+	1.0	64	69	67
	+	5.0	74	57	50
	+	10.0	38	50	43
	+	25.0	48	63	49

Table 435

IN VITRO ASSAYS WITH *ESCHERICHIA COLI* WP2 IN DESICCATORS - EIGHT-HOUR EXPOSURE

VINYL FLUORIDE

Experiment 2

Compound	Metabolic Activation	Percent of Compound in Desiccator	Tryptophan Revertants per Plate		
Negative Control	-		17	33	45
	+		49	56	43
Positive Control					
Ethylene oxide	-	0.06	451	434	511
	+	0.06	501	487	590
Vinyl fluoride	-	5.0	29	33	45
	-	10.0	40	48	54
	-	15.0	38	38	38
	-	25.0	30	30	20
	+	5.0	48	55	55
	+	10.0	51	59	52
	+	15.0	49	43	44
	+	25.0	45	37	30

Table 436

IN VITRO ASSAYS SACCHAROMYCES CEREVISIAE D3 IN DESICCATORS - 48-HOUR EXPOSURE

VINYL FLUORIDE

Experiment 1

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Mitotic Recombinants per Plate				
Negative Control	-		0	0	2	0	2
	-		2	1	2	0	1
	+		1	1	1	2	3
	+		1	1	1	0	0
Positive Control							
1,2,3,4-Dienoxybutane	-	0.02	115	110	110	92	107
	+	0.02	97	100	93	90	101
Vinyl Fluoride	-	1	2	0	4	7	0
	-	5	1	4	3	1	2
	-	10	2	3	0	4	0
	-	25	0	0	0	0	0
	+	1	2	4	2	5	2
	+	5	4	1	3	3	1
	+	10	2	2	0	0	2
	+	25	0	0	0	0	0

Table 437

IN VITRO ASSAYS SACCHAROMYCES CEREVISIAE D3 IN DESSICATORS - 48-HOUR EXPOSURE
VINYL FLUORIDE

Experiment 2

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Mitotic Recombinants per Plate				
Negative Control	-		3	4	5	3	2
	-		3	3	3	3	2
	+		1	5	3	0	4
	+		4	0	2	3	1
Positive Control							
1,2,3,4-Diepoxybutane	-	0.02	114	93	115	99	90
	+	0.02	91	82	120	78	106
Vinyl Fluoride	-	5	1	1	1	1	3
	-	10	3	1	3	3	1
	-	15	1	1	3	1	0
	-	25	3	1	3	4	1
	+	5	3	2	2	3	0
	+	10	1	1	2	1	1
	+	15	1	5	0	2	1
	+	25	2	1	2	2	4

REFERENCES

1. Short-Term Tests for Carcinogenesis. Report of the International Collaboration Program. F. J. deSerres and J. Ashby, Eds., Elsevier-North Holland, Amsterdam, 1980.

2. Mitchell, A. D., E. L. Evans, M. M. Jotz, E. S. Riccio, K. E. Mortelmans and V. F. Simmon. Mutagenic and Carcinogenic Potency of Extracts of Diesel and Related Environmental Emissions: In Vitro Mutagenicity and DNA Damage. In: Health Effects of Diesel Engine Emissions: Proceedings of an International Symposium, Volume 2. W. E. Petelko, R. M. Danner and N. A. Clarke, Eds., U.S. Environmental Protection Agency Document, EPA-600/9-80-057b, 1980, pp. 810-842.

3. McCann, J., E. Choi, E. Yamasaki, and B. N. Ames. Detection of Carcinogens as Mutagens in the Salmonella/microsome test: Assay of 300 Chemicals. Proc. Nat. Acad. Sci. USA 72, 5135-5139 (1975).

4. Mortelmans, K. E. Salmonella/mammalian-microsome test to detect chemical mutagens. J. Food Prot. 41, 989-995 (1978).

5. Ames, B. N., J. McCann, and E. Yamasaki. Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutat. Res. 31, 347-364 (1975).

6. McCann, J., N. E. Spingarn, J. Kobori, and B. N. Ames. Detection of Carcinogens as Mutagens: Bacterial tester strains with R factor plasmids. Proc. Nat. Acad. Sci. USA 72, 979-983 (1975).

7. Mortelmans, K. E., and B.A.D. Stocker. Segregation of the mutator property of plasmid R46 from its ultraviolet-protecting property. Mol. Gen. Genet. 167, 317-327 (1979).

8. Bridges, B. A. Simple bacterial systems for detecting mutagenic agents. Lab. Pract. 21, 413-416 (1972).

9. McCalla, D. R. and D. Voutsinos. On mutagenicity of nitrofurans. Mutat. Res. 26, 3-16 (1974).

10. Zimmermann, F. K., and R. Schwaier. Induction of mitotic gene conversion with nitrous acid, 1-methyl-3-nitro-1-nitrosoguanidine and other alkylating agents in Saccharomyces cerevisiae. Mol. Gen. Genet. 100, 63-69 (1967).

11. Brusick, D. J., and V. W. Mayer. New Developments in Mutagenicity Screening Techniques with Yeast. Environ. Health Perspectives 6, 83-96 (1973).

12. Zimmerman, F. K. Procedures used in the induction of mitotic recombination and mutation in the yeast Saccharomyces cerevisiae. Mutation Res. 31, 71-86 (1975).

13. Zimmerman, F. K. A yeast strain for simultaneous detection of induced mitotic crossing over, mitotic gene conversion, and reverse mutation. Mutation Res. 28, 381-388 (1976).

14. DeLucia, P. and J. Cairns. Isolation of an E. coli strain with a mutation affecting DNA polymerase. Nature 224, 1164-1166 (1969).

15. Kelly, R. B., M. R. Atkinson, J. A. Huberman, and A. Kornberg. Excision of thymine dimers and other mismatched sequences by DNA polymerase of Escherichia coli. Nature 224, 495-501 (1969).

16. Slater, E. E., M. D. Anderson, and H. S. Rosenkranz. Rapid Detection of Mutagens and Carcinogens. Cancer Res. 31, 970-973 (1971).

17. Kada, T. Mutagenicity Testing of Chemicals in Microbial Systems. In: New Methods in Experimental Chemistry and Toxicology. F. Coulston, F. Corte and M. Coto, Eds. International Academic Printing. Tokyo, 1973, pp. 127-133.

18. MacPhee, D. G. Recombination-Deficient Mutants of Colicinogenic Salmonella typhimurium detected by their failure to produce colicin. J. Bacteriol. 104, 345-350 (1970).

19. Kato, H. Induction of Sister Chromatid Exchanges by Chemical Mutagens and Its Possible Relevance to DNA Repair. Exp. Cell Res. 85, 239-247 (1974).

20. Perry, P., and H. J. Evans. Cytological Detection of Mutagen-Carcinogen Exposure by Sister Chromatid Exchange. Nature 158, 121-125 (1975).

21. Solomon, E., and M. Bobrow. Sister Chromatid Exchanges--A Sensitive Assay of Agents Damaging Human Chromosomes. Mutat. Res. 30, 273-278 (1975).

22. Wolff, S. 1976. Technique for Obtaining Harlequin Chromosomes. Mammalian Chromosome Newsletter 17, 26 (1976).

23. Natarajan, A. F., A. D. Tates, P.P.W. van Buul, M. Meijers, and N. de Vogel. Cytogenetic Effects of Mutagens/Carcinogens After Activation in a Microsomal System in vitro. 1. Induction of Chromosome Aberrations and Sister Chromatid Exchanges by Diethylnitrosamine (DEN) and Dimethylnitrosamine (DMN) in CHO Cells in the Presence of Rat-Liver Microsomes. Mutat. Res. 37, 83-90 (1976).

24. Stetka, D. G., and S. Wolff. Sister Chromatid Exchanges as an Assay for Genetic Damage Induced by Mutagens/Carcinogens. Part II. In vitro Test for Compound Reequiring Metabolic Activation. Mutat. Res. 41, 343-350 (1976).

25. Wolf, S. Sister Chromatid Exchange. Ann. Rev. Genet. 11, 183-201 (1977).

26. Mitchell, A. D., T.J.A. Kan, C. F. Kirchner, P. A. McAfee, and D. E. Robinson. The Effects of $Pt(SO_4)_2$ on the Induction of Sister Chromatid Exchanges and on Unscheduled DNA Synthesis. J. Cell Biol. 75, No. 2, Part 2, 125 (1977).

27. Mitchell, A. D., D. E. Robinson, D. T. Tajiri, T.J.A. Kan, G. P. Sterling, P. A. McAfee, and C. F. Kirchner. The Relationship of Unscheduled DNA Synthesis to Mutagenesis and Chromosomal Aberrations. Mutat. Res. 53, 231a (1978).

28. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. Protein Measurement with the Folin Phenol Reagent. J. Biol. Chem. 193, 265-275 (1951).

29. Van Cantfort, J., J. DeGraeve, and J. E. Gielsen. Radioactive Assay for Aryl Hydrocarbon Hydroxylase. Improved Method and Biological Importance. Biochem. Biophys. Res. Commun. 79, 505-512 (1977).

30. Clive, D., W. G. Flamm, M. R. Machesko, and N. J. Bernheim. A Mutational Assay System Using the Thymidine Kinase Locus in Mouse Lymphoma Cells. Mutat. Res. 16, 77-87 (1972).

31. Clive, D., W. G. Flamm, and J. B. Patterson. Locus Mutational Assay Systems for Mouse Lymphoma Cells. In: Chemical Mutagens: Principals and Methods for Their Detection, Vol. 3. A. Hollaender, Ed., Plenum Press, New York, 1973, pp. 79-103.

32. Clive, D., and J.F.S. Spector. Laboratory Procedure for Assessing Specific Locus Mutations at the TK Locus in Cultured L5178Y Mouse Lymphoma Cells. Mutat. Res. 31, 17-29 (1975).

33. Clive, D., and P. Voytek. Evidence for Chemically Induced Structural Gene Mutations at the Thymidine Kinase Locus in Cultured L5178Y Mouse Lymphoma Cells. Mutat. Res. 44, 269-278 (1977).

34. Brown, M.M.M., and D. Clive. 1978. The Utilization of Trifluorothymidine as a Selective Agent for $TK^{-/-}$ Mutants in L5178Y Mouse Lymphoma Cells. Mutat. Res. 53, 116 (1978).

35. Clive, D., K. O. Johnson, J.F.S. Spector, A. G. Batson, and M.M.M. Brown. Validation and Characterization of the L5178Y/$TK^{+/-}$ Mouse Lymphoma Mutagen Assay System. Mutat. Res. 59, 61-108 (1979).

36. Rasmussen, R. E. and R. B. Painter. Radiation-Stimulated DNA Synthesis in Cultured Mammalian Cells. J. Cell Biol. 29, 11-19 (1966).

37. Berky, J. and C. Sherrod. In Vitro Toxicity Testing. Franklin Institute Press, Philadelphia, 1978, pp. 336-343.

38. Schmidt, G. and S. J. Thannhauser. A Method for the Determination of Deoxyribonucleic Acid, Ribonucleic Acid, and Phosphoproteins in Animal Tissues. J. Biol. Chem. 161, 83-89 (1945).

39. Richards, G. M. Modification of the Diphenylamine Reaction Giving Increased Sensitivity and Simplicity in the Estimation of DNA. Anal. Biochem. 57, 369-374 (1974).

40. Schmid, W. The Micronucleus Test for Cytogenetic Analysis. In Chemical Mutagens, Vol. 4. A. Hollaender, Ed., Plenum Press, New York, 1976, pp. 31-53.

41. Mackey, B., and J. MacGregor. The Micronucleus Test: Statistical Design and Analysis. Mutat. Res. 64, 195-204 (1979).

42. Valencia, R. Mutagenesis Screening of Pesticides using *Drosophila*. WARF Institute, Inc., Madison, Wisconsin. Final Report on Contract #68-01-2474, U.S. Environmental Protection Agency, 1977.

43. Murnik, M. R., and C. L. Nash. Mutagenicity of the Triazine Herbicides Atrazine, Cyanazine, and Simazine in *Drosophila melanogaster*. J. Tox. Env. Health 3, 691-697 (1977).

44. Magnusson, A., C. Ramel and A. Ericksson. Mutageanic Effects of Chlorinated Phenoxyacetic Acids in *Drosophila melanogaster*. Hereditas 87, 121 (1977).

45. Würgler, F. E., F. H. Sobels, and E. Vogel. *Drosophila* as Assay System for Detecting Genetic Changes. In Handbook of Mutagenicity Test Procedures, B. J. Kilbey, M. Legator, W. Nichols, and C. Ramel, Eds. Elsevier/North Holland Biomedical Press, Amsterdam, 1977, pp. 335-373.

46. Lindsley, D. L., and E. H. Grell. Genetic Variations of *Drosophila melanogaster*. Carnegie Institute, Washington, D.C., Publ. No. 627 (1968).

47. Chandley, A. C., and A. J. Bateman. Timing of Spermatogenesis in *Drosophila melanogaster* Using Tritiated Thymidine. Nature 193, 299-300 (1962).

48. Würgler, F. D., U. Graf, and W. Berchtold. Statistical Problems Connected with the Sex-Linked Recessive Lethal Test in *Drosophila melanogaster*. I. The Use of the Kastenbaum-Bowman Test. Arch. Genet. 48, 158-178 (1975).

49. Engels, W. R. The Estimation of Mutation Rates When Premeiotic Events are Involved. Environ. Mutagen. 1, 37-43 (1979).

50. Kastenbaum, M. A., and K. O. Bowman. Tables for Determining the Statistical Significance of Mutation Frequencies. Mutat. Res. 9, 527-549 (1970).

51. Simmon, V. F., A. D. Mitchell and T. A. Jorgenson. Evaluation of Selected Pesticides as Chemical Mutagens. *In vitro* and *in vivo* studies. U.S. Environmental Protection Agency Document EPA-600/1-77-028 (1977).

52. Simmon, V. F. *In vitro* Microbiological Mutagenicity and Unscheduled DNA Synthesis Studies of Eighteen Pesticides. Final Report--Phase II, Contract No. 68-01-2458, U.S. Environmental Protection Agency, 1978.

53. Simmon, V. F., E. S. Riccio, D. E. Robinson and A. D. Mitchell. In vitro Microbiological Mutagenicity and Unscheduled DNA Synthesis Studies of Fifteen Pesticides. Final Report--Phase III, Contract No. 68-01-2458, U.S. Environmental Protection Agency, 1979.

54. Simmon, V. F., E. S. Riccio and A. Griffin. In vitro Microbiological Mutagenicity Assays of Diallate and Triallate. Final Report, Contract No. 68-01-2458, U.S. Environmental Protection Agency, April 1978.

55. Vogel, E. and F. H. Sobels. The Function of Drosophila in Genetic Toxicology Testing. In: Chemical Mutagens, Vol. 4, A. Hollaender, Ed., Plenum Publishing Corp., New York, 1976, pp. 93-142.

56. Vogel, E. and J.L.R. Chandler. Mutagenicity Testing of Cyclamate and Some Pesticides in Drosophila melanogaster. Specialia 15, 6 (1974).

57. Seiler, J. P. Herbicidal Phenylalkylureas as Possible Mutagens. I. Mutagenicity Tests With Some Urea Herbicides. Mutat. Res. 58, 353-359 (1978).

58. Hite, M., M. Pecharo, I. Smith, and S. Thornton. The Effect of Benzene in the Micronucleus Test. Mutat. Res. 77, 149-155 (1980).